The Journal of
CLINICAL
ENDOCRINOLOGY

VOLUME 4 MARCH, 1944 NUMBER 3
Copyright 1944 by the Association for the Study of Internal Secretions

A Correlation of the Ovarian and Endometrial Histology, Vaginal Epithelium, Gonadotrophic Hormonal Excretion and the Day of the Menstrual Cycle in 28 Women[1]

CARL G. HELLER,[2] M.D., Ph.D.,
JACOB P. FARNEY, M.D.,
DONALD N. MORGAN, M.D., AND
GORDON B. MYERS, M.D.

From the Department of Medicine, Wayne University College of Medicine, and the Medical and Gynecological Services of Receiving Hospital, Detroit, Michigan

NUMEROUS attempts have been made to predict the functional state of the ovaries and the time of ovulation from one or more of the following criteria: the day of the menstrual cycle, the predominating cell in the vaginal smear, the histological structure of the endometrium and the amount of gonadotrophic hormone excreted in the urine. The purpose of this communication is to evaluate each of the foregoing criteria by correlation with the gross and histological appearance of the ovaries. The studies were carried out on 28 women, of ages 19 to 58 years, upon whom bilateral salpingo-oöphorectomy and hysterectomy were performed because of chronic salpingitis or fibromyomata.

PROCEDURE

In each case, both ovaries and uterus were removed, preserved in formalin and submitted to gross and histological examination. On the day prior to the operation vaginal smears were obtained by using a dry pipette and suction bulb. The slides were fixed and preserved in a

[1] Aided by a grant from Frederick Stearns and Company, administered by Dr. Richard M. Johnson, Medical Director.
[2] Research Fellow, American College of Physicians.

solution consisting of equal parts of ether and ethyl alcohol and were subsequently stained by Leishman's method. The smears were classified into five main categories[3] on the basis of cell distribution and morphology as follows. Four plus indicated full cornification. Three plus represented a preponderance of non-cornified cells with a minority of fully cornified cells. Two plus included two varieties: a) smears consisting almost exclusively of non-cornified cells with pyknotic nuclei; b) smears with a predominance of the above cells accompanied by a minority of cells with vesicular nuclei and perhaps an occasional cornified cell. One plus also included two subdivisions: a) smears consisting almost exclusively of cells with vesicular nuclei; and b) smears with a majority of such cells, accompanied by a minority of non-cornified cells with pyknotic nuclei and perhaps an occasional deep cell. Zero included all smears with an appreciable number of deep cells. In using the Leishman stain, the principal criterion for classifying smears is cell morphology rather than color reaction as in the methods developed by Shorr (1). In all cases, duplicate smears were stained by the iodine method of Mack (2) and compared with the Leishman stained sections. With this method we could distinguish between zero and four plus smears in most instances, but could not make dependable differentiations in the intermediate categories.

Urinary gonadotrophic excretion was determined on the specimen collected during the 12-hour over-night period prior to operation by precipitating with 95% ethyl alcohol, subsequently dialyzing off toxic products and reprecipitating with 95% ethyl alcohol (3). The final precipitate evolving from this procedure was taken up in 6 cc. of tap water and injected into a 22–24-day-old female albino rat[4] in 1 cc. portions twice daily for three days. The animal was killed 16–24 hours after the last injection and the vaginal canalization was noted. This was not nearly as sensitive an index as uterine and ovarian weight. The ovaries were weighed in pairs and the uterus was weighed before and after expressing the intrauterine fluid. It was found expedient to express gonadotrophic activity in terms of the actual ovarian weights elicited by the concentrate of a single 12-hour over-night specimen of urine injected into one rat. Normal ovarian weights for the strain of animals used ranged from 8 to 16.2 mg. and averaged 12 mg. Assays of single 12-hour specimens from 28 post-menopausal and castrated women invariably gave ovarian weights above 40 mgm. and usually gave weights within the range of 60 to 110 mgm. This is referred to as "high" excretion. The ovaries from the rats receiving concentrates of castrate or post-menopausal urine often showed luteinization as well as follicular growth. Evidence of progestational activity was demonstrable in the uteri in instances where the ovaries were luteinized. These findings indicated an effect of interstitial-cell-stimulating hormone (ICSH), which is also known as luteinizing hormone (LH), in addition to an effect of the gametogenic hormone or follicle stimulating hormone (FSH). When concentrates of single 12-hour specimens from normal women were used, ovarian weights were invariably below 40 mgm. In many instances, they were below 16.2 mgm. which is the upper limit of normal in uninjected control rat ovaries The presence of gonadotrophins in such urines was often indicated by uterine stimulation alone (4). This is referred to as "low" excretion. In the remaining normals the assay gave ovarian weights between 16.2 and 33 mgm. This is referred to as "peak" excretion. The increased ovarian weight in the "peak" group was due entirely to follicular growth. From unpublished data (5) the simultaneous administration of purified pituitary FSH and ICSH, in amounts which were insufficient to affect ovarian and uterine weight when given separately, produced follicular growth and estrogenic uterine stimulation. Greep et al (6) have shown that purified FSH is without effect upon uterine growth unless minute amounts of ICSH are added. The gross effects of the FSH-ICSH combination

[3] In the graphic charts accompanying the companion articles the last three categories are each broken down into three sub-groups, in order to depict minor as well as major changes.

[4] Use of a single rat as the recipient for the entire concentration was justified by the marked difference in the response between urines from normal and menopausal women. These differences were much greater than the variations between animals. The use of a single rather than several rats for each assay permitted serial studies of a larger group of cases. Actual ovarian weights are recorded instead of arbitrary units in order to facilitate comparisons with other laboratories.

TABLE 1

Group	Case No.	Ovaries Gross	Ovaries Microscopic	Endometrial Histology	Vaginal Smear	Urinary Gonadotrophins		Day of Cycle	Age of Patient
						Classed as:	Recipient Rat Ovarian Wt. Mg.		
1 A	1	Atrophic	Atrophic	Atrophic	0	High	87	3 years	50
	2	Atrophic	Atrophic	Atrophic and cystic	0	High	70	8 years	57
1 B	3	Atrophic	Atrophic	Hypoplastic	+	High	45	6	32
	4	Atrophic	Atrophic	Hypoplastic	++	High	123	Cont. flow —21	42
	5	Atrophic	Atrophic	Atrophic	+	High	84	28	36
2	6	Normal with hem. cyst	Follicles and regressing C. L.	Late prolif.	++	Peak	24	16	32
	7	Left—normal Rt.—atrophic	Large follicles and regressing C. L.	Prolif.	+++	Low	10	24	30
3	8	Normal	Corp. hemorrhagicum	Late prolif.	++	Low	10	15	44
	9	Left—normal Rt.—atrophic	Corp. hemorrhagicum	Early secretory	++	Peak	23	11	32
	10	Normal	Corp. hemorrhagicum	Early secretory	++	Low	14	13	42
	11	Normal	Late corpus hemorrhagicum	Early secretory	++	Peak	26	19	36
4 A	12	Normal	Early C. L.	Early secretory	+++	Low	14	11	48
	13	Normal	Early C. L.	Early secretory	++	Low	9	16	34
	14	Normal	Early C. L.	Early secretory	+++	Peak	19	21	31
	15	Normal	Early C. L.	Early secretory	++	Peak	22	17	39
	16	Cystic-bilat.	Abscess-bilat.	Early secretory	+++	Peak	33	17—irreg.	22
4 B	17	Normal	Incomplete	Mid-secretory	++	Low	9	24	41
	18	Normal	Mid C. L.	Mid-secretory	++	Low	13	24	42
	19	Normal	Mid C. L.	Missing	++	Low	15	22	33
	20	Normal	Late C. L.	Mid-secretory	++	Low	15	21	36
	21	Normal	Late C. L.	Mid-secretory	+++	Low	10	25	24
	22	1—normal 1—fibrotic	— Late C. L. early deg.	Late-secretory	++	Low	9	26	36
	23	Abscess	Late C. L.	Late-secretory		Peak	30	14 metrorrhagia	19
	24	1—normal 1—abscess	Late C. L.	Menstruating	+	Low	10	3	36
5	25	Cystic	Follicular cysts	Late secretory	++	Low	11	Cont. flow 5 wks.	45
	26	Cystic	Follicular cysts	¾ secretory, ¼ prolif.	+	Low	10	31	24
	27	Normal	Hem. C. L. cyst	Early prolif. & deg. placental tissue	+++			51	34
	28[1]	Cystic	Hem. C. L. cyst	Hypoplastic	+++	Low	16	17	26

[1] Resection of ovaries.

were thus similar to the "peak" responses obtained from concentrates of urine from normal women. Since the measurable effects upon the ovaries and uteri in the "peak" range and in the "high" range could be attributed to the combined effects of FSH and ICSH the assay was considered a measure of "gonadotrophic" hormone.

RESULTS

The essential data are summarized in table 1. The cases were classified into the following five main groups according to the gross and microscopic appearance of the ovaries: 1) atrophic; 2) pre-ovulatory, containing follicles but no functioning corpus luteum; 3) ovulatory or immediately post-ovulatory as indicated by an early hemorrhagic corpus luteum; 4) post-ovulatory associated with well-developed corpus luteum; and 5) atypical.

Group 1 included all cases with atrophic

ovaries. The five cases so classified could be further subdivided by menstrual history into A) those beyond the menopause (*Cases 1* and *2*); and B) those in whom menstrual bleeding still occurred (*Cases 3–5*). *Cases 1* and *2* were the oldest in the series (ages 50 and 57 years) and had had no menses for three and eight years respectively. The ovaries and endometrium were grossly and microscopically atrophic. Vaginal smears also indicated complete atrophy. Urinary gonadotrophins were unquestionably "high." *Cases 3–5* also had atrophic ovaries and atrophy or hypoplasia of the endometrium. Vaginal smears indicated low estrogenic activity in *Cases 3* and *5*. *Case 4*, who had continuous vaginal bleeding from uterine fibroids for 21 days prior to the operation, exhibited a two plus smear, which was probably produced by the mechanical irritation consequent upon continuous bleeding.

Thus *Cases 3*, *4* and *5* were similar to the two post-menopausal women in urinary gonadotrophin titer and in the histological state of the ovaries and uterus. They differed principally in regard to vaginal bleeding and slightly in the character of the vaginal smear. Even though these patients still had vaginal bleeding it is evident from the study of the ovaries that they had already entered the menopause. The presence of intermittent vaginal bleeding does not necessarily indicate that the ovaries are functioning normally. The bleeding in *Case 4* was attributed to the fibromyomata and in *Cases 3* and *5* might have been associated with the pelvic inflammatory disease. On the other hand, the ovarian atrophy could be accurately predicted from the endometrial histology or from the fact that urinary gonadotrophins were in the menopausal range.

Group 2 included two cases who were in the pre-ovulatory phase of the cycle as indicated by the presence of follicles and absence of functioning corpora lutea. Regressing corpora lutea were found, indicating previous cyclic changes. The endometrium in both instances was in the proliferative phase and the vaginal smears indicated normal estrogenic activity. In *Case 6*, operation was performed on the sixteenth day of the menstrual cycle. The peak of gonadotrophin excretion which was encountered in this case may have been associated with the imminence of ovulation. *Case 7*, operated upon during the twenty-fourth day of the cycle, probably represents an example of an anovulatory cycle. Gonadotrophins were low.

Group 3 included four cases who had recently ovulated as shown by the presence of hemorrhagic corpora lutea. *Cases 8*, *9*, and *10* had early corpora hemorrhagica and *Case 11* had a late corpus hemorrhagicum. In *Case 8* the endometrium was still proliferative in character. This probably represented a lag between actual ovulation, which had already occurred, and response of the endometrium to progesterone, which had not as yet taken place. The other three had early secretory type endometria. All showed signs of estrogenic activity in the vaginal smear but none showed the cornification reputedly encountered at about this time. Peak excretion of gonadotrophins occurred in two cases, and low titers in the other two.

Group 4 included thirteen cases who had well formed corpora lutea indicating they were in the post-ovulatory phase of the cycle. These cases could be further subdivided into A) those with recently formed corpora lutea, B) those with older corpora lutea. Early secretory endometria were associated with the recently formed corpora lutea, whereas middle or late secretory endometria were associated with older corpora lutea. The vaginal smears in both sub-groups showed normal estrogenic stimulation except for *Case 24*, who was menstruating. Peaks of gonadotrophic hormone excretion occurred three times in the early secretory phase and once during the late secretory phase, the remainder having low titers.

Atypical cases were classified in Group 5. The four cases included in this group had cystic ovaries, two showing follicular cysts and two hemorrhagic corpus luteum cysts. No correlation could be established between the ovarian histology, endometrial histology and vaginal smear. Two had menstrual irregularities and two did not. It has been claimed that cystic ovaries may be produced by excessive doses of pituitary gonadotrophins. Our own clinical experience with the various commercial[1] anterior pituitary preparations has indicated that most are relatively inert. Thus it seems unlikely that over-stimulation could be produced

by them. Therefore it was of interest to us that all three patients upon whom gonadotrophin assays were performed showed low titers.

DISCUSSION

Our results indicate that the best single criterion of the histological state of the ovaries is the endometrial histology. The endometrium was atrophic or hypoplastic in all five cases with non-functional ovaries; was proliferative during the pre-ovulatory period; was either late proliferative or early secretory during the ovulatory or immediate post-ovulatory period; and was middle or late secretory or menstrual in women whose ovaries contained older corpora lutea. The endometrium was of no aid in predicting the occurrence of cystic ovaries, although in three of four cases it was atypical in one respect or another.

The vaginal smear picture failed to indicate the phase of the ovarian cycle. Two plus and three plus activity occurred in smears taken both in the pre-ovulatory and the post-ovulatory phase of the cycle. The smear exhibiting the lowest activity during the cycle occurred at or near the menses. Smears tended to reflect the low ovarian activity in the group with atrophic ovaries. The conclusions regarding the smears cannot be considered conclusive since the patients were afflicted with gynecological disorders which conceivably may have modified the response.

A study of the urinary gonadotrophins will definitely separate cases with non-functioning ovaries from cases with functioning ovaries. The titer in all patients with ovarian atrophy was significantly higher than in any case with functional ovaries. The high titers in ovarian atrophy are probably an expression of the failure of these ovaries to utilize the hormone which consequently spills over in the urine.

On the other hand, these studies show that the stage of the ovarian cycle in patients with functioning ovaries could not be predicted from the amount of gonadotrophic hormone in the urine. It has been claimed that the occurrence of ovulation is indicated by a peak of excretion (D'Amour 7, 8). While a higher percentage of peak excretions were obtained in our cases who were studied near the time of ovulation, it can be seen from table 1 that peaks were encountered during the proliferative, ovulatory, early secretory and late secretory phases of the cycle. It can be seen also that low excretion occurred during each phase. These findings are in keeping with those of Emily Heller (9), Werner (10, 11), and Main et al (12), who found that peaks may occur at any time of the normal cycle except during menstruation.

The day of the menstrual cycle serves only as a wide and vague reference point in predicting the phase of the menstrual cycle.

SUMMARY

In an attempt to ascertain the most reliable clinical criterion of the histological state of the ovaries, a study of the endometrial histology, vaginal smears, and gonadotrophic hormone excretion was made in 28 women upon whom bilateral salpingo-oöphorectomy and hysterectomy were performed.

The vaginal smears and the day of the menstrual cycle were found to be unreliable indices of the state of the ovaries. From the titer of urinary gonadotrophins, cases with non-functioning ovaries could be accurately differentiated from those with functioning ovaries but the stage of the ovarian cycle and the time of ovulation could not be predicted. The best single clinical criterion for judging the state of the ovaries proved to be the endometrial histology.

A correlation between the histological state of the ovaries and uterus was obtained in all but four cases, who showed cystic ovaries. Low titers of gonadotrophins were associated with cystic ovaries.

REFERENCES

1. SHORR, E.: New technic for staining vaginal smears; single differential stain. *Science* 94: 545, 1941.
2. MACK, H. C.: New and rapid method of staining vaginal smears based upon specific color reaction for glycogen. *Harper Hosp. Bull.* 1: 54. 1942.
3. HELLER, C. G., AND R. E. CHANDLER: Gonadotropic hormone; modification of the alcohol-precipitation assay method. *J. Clin. Endocrinol.* 2: 252. 1942.
4. HELLER, C. G., H. D. LAUSON AND E. L. SEVRINGHAUS: The immature rat uterus as an assay endpoint for gonadotropic substances. *Am. J. Physiol.* 121: 364. 1938.
5. MCSHAN, H., H. D. LAUSON AND C. G. HELLER: Unpublished data.
6. GREEP, R. O., H. B. VAN DYKE AND B. F. CHOW: Gonadotropins of swine pituitary; various bio-

logical effects of purified thylakentrin (FSH) and pure metakentrin (ICSH). *Endocrinol.* 30: 635. 1942.
7. D'AMOUR, F. E.: Comparisons of methods used in determining the time of ovulation. *J. Clin. Endocrinol.* 3: 41. 1943.
8. D'AMOUR, F. E.: Further studies on hormone excretion during the menstrual cycle. *Am. J. Obst. & Gynec.* 40: 958. 1940.
9. HELLER, E. J.: Gonadotropic hormone; urine assays during the menstrual cycle in normal women. *J. Clin. Endocrinol.* 1: 813. 1941.
10. WERNER, S. C.: Quantitative study of urinary excretion of hypophyseal gonadotropin, estrogen, and androgen of normal women. *J. Clin. Investigation* 20: 21. 1941.
11. WERNER, S. C.: Quantitative study of urinary excretion of hypophyseal gonadotropin, estrogen, and neutral 17-ketosteroids of normal men. *J. Clin. Investigation* 22: 395. 1943.
12. MAIN, R., W. COX, R. O'NEAL AND J. STOECKEL: Gonadotropin excretion in normal men and women and cases of hysterectomy, menopause, migraine, epilepsy and eunuchoidism. *J. Clin. Endocrinol.* 3: 331. 1943.

Development and Correlation of Menopausal Symptoms, Vaginal Smear and Urinary Gonadotrophin Changes Following Castration in 27 Women[1]

CARL G. HELLER,[2] M.D., Ph.D.,
JACOB P. FARNEY, M.D., AND
GORDON B. MYERS, M.D.

From the Department of Medicine, Wayne University College of Medicine, and the Medical and Gynecological Services of Receiving Hospital, Detroit, Michigan

SIMULTANEOUS clinical studies and urinary gonadotrophin assays were conducted before and after bilaterals alpingo-oöphorectomy and hysterectomy in 27 women suffering from chronic pelvic inflammatory disease and/or uterine fibromyomata. Correlations made at the time of operation between the gonadotrophic titers, vaginal smears, endometrial structure and ovarian histology have been reported (1). In this communication, post-castrational changes in symptomatology, vaginal cytology and gonadotrophic output are described and correlated.

PROCEDURE

Following castration frequent periodic observations were made of menopausal symptoms, vaginal smears and urinary gonadotrophic output. Urine for gonadotrophin assays was collected during the 12-hour over-night period before each interview with the patient. Vaginal smears were obtained at the time of the interview. The technic of urine assay and the preparation of the vaginal smears were described in the previous article (1). These studies were made on both the sixth and tenth post-operative days in 25 cases, on the sixth day in one of the remainder, and on the tenth day in the other case. Subsequent interviews were planned at two-week to three-week intervals, but absenteeism occurred in some instances. Twenty-five patients reported at least once between the twenty-fifth and thirty-fourth post-operative days, one of the remainder reported on the fortieth day, and the other did not appear until the one-hundred-third day. Studies were repeated, at least once, between the thirty-ninth and fifty-sixth days in 24 cases, and again between the fifty-eighth and sixty-ninth days in 22 cases. Treatment of the menopausal symptoms was withheld until adequate observations of the post-castration changes could be made and the menopausal syndrome was firmly established. An average of 5.5 observations was made after castration and before the institution of therapy. The significant data are recorded in table 1. The case numbers in this table correspond to those of table 1 of the companion article (1).

SYMPTOMS

Development of symptoms. At each interview specific inquiries were made regarding the presence and severity of the following symptoms: hot flashes, sweating, palpitation, nervousness, irritability, fatigue, early morning insomnia, arthralgia, vertigo, headache, depression, self-depreciation, crying spells and suicidal tendencies. On the basis of the number and severity

[1] Aided by a grant from Frederick Stearns & Company, administered by Richard M. Johnson, Medical Director.
[2] Research Fellow, American College of Physicians.

of symptoms, cases were graded from zero to four plus. No patient had more than one of these symptoms before oöphorectomy and no new symptoms were reported at the interviews on the sixth and tenth post-operative days. Definite menopausal symptoms were present in all but four of the 25 cases observed between the twenty-fifth and thirty-fourth post-operative days. Symptoms developed in three of the remaining four patients sometime between the fifty-fifth and sixty-sixth days. The fourth patient was symptom free when last observed on the forty-fourth post-operative day. Of the two patients who did not return for interview between the twenty-fifth and thirty-fourth days, one reported symptoms on the fortieth and the other reported minimal symptoms when seen on the one-hundred-third day. Thus menopausal symptoms developed in all patients except in *Case 5*, who disappeared from observation after the forty-fourth day. *Case 5* had atrophic ovaries and uterus and high urinary gonadotrophins at the time of operation. However, definite menopausal symptoms developed in the other four cases where atrophic ovaries and uteri and high gonadotrophins were found at castration. This included *Cases 1* and *2* who had undergone spontaneous menopause three years and eight years previously. The development and course of the symptoms in *Cases 2*, *3* and *4* are illustrated in figures 1, 2, 3[3] respectively. The symptoms which followed the removal of the atrophic ovaries were of moderate severity in *Cases 2* and *4* and were mild in *Cases 1* and *3*.

TABLE 1

Group	Case No.	Urinary Gonadotrophins (Recipient Rat Ovarian Wt. (Mg.)					Vaginal Smear				Symptoms			
		Pre-op.	Post-operative Day			Max.	Pre-op. Grade	Day of First Retrogression	Lowest Grade	Day of Maximal Retrogression	Pre-op. Grade	Day of Appearance	Maximal Grade	Day Maximum Established
			6	10	25-34									
1 A	1	87	65	88	85	88	0	—	0	—	0	34	+	34
	2	70	60	55	—	123	0	—	0	—	0	34	++	34
1 B	3	45	36	63	49	63	+	27	0	27	0	27	+	27
	4	123	67	68	67	89	++	31	+	31	0	66	++	66
	5	84	84	80	49	84	+	10	0	27	0	—	0	—
2	6	24	55	39	53	73	++	6	+	55	0	26	++	69
	7	10	10	64	58	64	+++	6	++	6	0	27	++	27
3	8	10	67	87	168	168	++	26	0	61	0	26	+	26
	9	23	66	65	58	66	++	6	+	6	0	25	++++	39
	10	14	—	62	—	62	++	—	++	—	0	40	+++	40
	11	26	15	58	—	58	++	—	++	—	0		+	103?
4 A	12	14	50	58	55	58	+++	41	++	41	0	27	++	41
	13	9	21	72	69	91	++	64	+	64	0	26	++	89
	14	19	—	42	66	66	+++	6	++	6	0	27	+++	45
	15	22	63	—	93	93	++	—	++	—	0	25	++++	53
	16	33	76	33	70	76	+++	6	+	41	0	55	+	55
4 B	17	9	114	34	75	114	++	6	+	27	0	27	++++	69
	18	13	31	38	96	96	++	25	+	25	0	33	++++	33
	19	15	85	56	113	138	++	—	++	—	0	34	++	68
	20	15	58	50	78	86	++	—	++	—	0	27	++	41
	21	10	39	31	43	89	+++	6	+	27	0	27	++++	27
	22	9	19	96	—	96	++	—	++	—	0	27	+	27
	23	30	47	98	71	98					0	27	+	27
	24	10	11	55	83	83	+	—	+	—	0	27	++++	83
5	25	11	7	17	71	80	++	6	0	28	0	28	++	42
	26	10	40	68	42	68	+	10	0	40	0	26	++	68
	27	—	39	58	50	84	+++	26	0	40	0	64	+	64

Mode of onset. A fulminant onset of the full-blown menopausal syndrome occurred in *Cases 18* and *21*. The symptoms did not increase

[3] The ovarian weights in the charts are recorded against a logarithmic scale expressed in milligrams. The line bisecting the charts marked "normal ovarian weight" indicates the upper limit of ovarian weight in unstimulated control assay rats.

further in these two cases during a period of 16 and 56 days respectively, which elapsed before institution of therapy. *Case 18* (fig. 4) was symptom-free on the twenty-fifth post-operative day and had symptoms of four plus severity on the thirty-third post-operative day. *Case 10*, who was not seen between the tenth and fortieth days, reported symptoms graded as three plus on the latter day. In ten other cases, symptoms proved to be at their maximum on the day they were first reported, despite an average interim of 39 days which elapsed between the time of their appearance and the institution of therapy. The onset was more gradual in the remaining 12 cases. The symptoms at the onset were classed as moderate in three cases and increased to severe in an average period of 29 days. The symptoms at the onset were classed as mild in nine cases and increased to severe in two cases (example: *Case 15*, fig. 5) in an average period of 42 days, and increased to moderate in seven cases (example: *Case 13*, fig. 6) in an average period of 32 days. Thus, menopausal symptoms which develop after castration generally make their appearance by the end of the first month and usually attain a plateau during the second month after operation.

TABLE 2. MENOPAUSAL SYMPTOMS

	At Onset	At Height
Number of patients	24	27
Hot flashes	20	22
Nervousness	18	22
Morning insomnia	9	17
Arthralgia	2	5
Palpitation	2	5
Sweating	17	23
Vertigo	8	13
Headache	10	16
Depression	8	12
Self depreciation	3	5
Crying spells	4	7
Irritability	4	9
Fatigue	2	7
Suicidal tendency	0	0

Nature of the symptoms. The incidence of the individual symptoms at the time of clinical appearance of the menopause and at its height are given in table 2. The commonest complaints were hot flashes, nervousness and sweating, followed by headache, morning insomnia, and vertigo. The relative incidence of the various symptoms was similar at the height of development to that at the onset of the menopausal syndrome.

An appreciable number showed depression and crying spells, but in no case were these symptoms severe enough to warrant the diagnosis of involutional melancholia. An underlying mental instability may be a prerequisite for a menopausal psychosis. Among 11 women who were questioned regarding libido, nine reported normal libido before operation. Libido was unchanged in three and decreased or disappeared in six.

VAGINAL CYTOLOGY

Transient stimulation was observed in all five cases in whom atrophic ovaries and endometria were found at operation and is illustrated by *Cases 2* and *3* (fig. 1, 2). This effect was demonstrable on the sixth to tenth post-operative day in four cases and on the twenty-seventh day in the other. In each instance, stimulation was followed by regression to a grade corresponding with the pre-operative level or below it. Transient stimulation on the twenty-seventh post-operative day was observed in two other women (*Cases 22* and *24*) with subsequent return to the pre-operative pattern. No significant change in the vaginal cytology was observed in five other cases, in all of whom the pre-operative smear was graded as two plus (example: *Case 13*, fig. 6).

Definite retrogression took place in the other 14 cases studied. It was observed as early as the sixth day and attained a maximum some time between the sixth and sixty-fourth days, most commonly at the end of the first month. As would be expected, retrogression was most rapid in the six cases whose pre-operative smears were graded the highest (three plus). In four of these significant retrogression was found by the sixth day (example: *Case 7*, fig. 7), whereas in the other two it was not observed until the twenty-sixth and forty-first days respectively. The retrogression stopped at grade two plus in three (example: *Case 7*, fig. 7), proceeded to one plus in two (example: *Case 16*, fig. 8), and went to zero in one (example: *Case 27*, fig. 9). Very slight retrogression was observed within six days in four of the remaining seven cases whose pre-operative smears

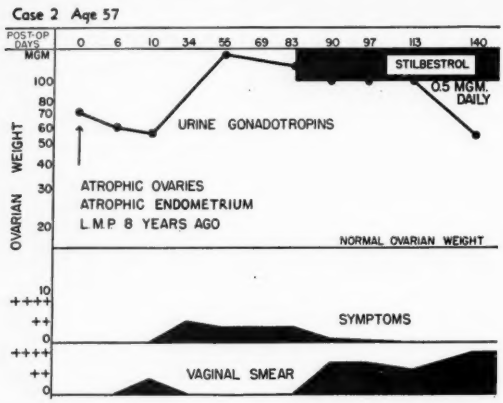

FIG. 1.

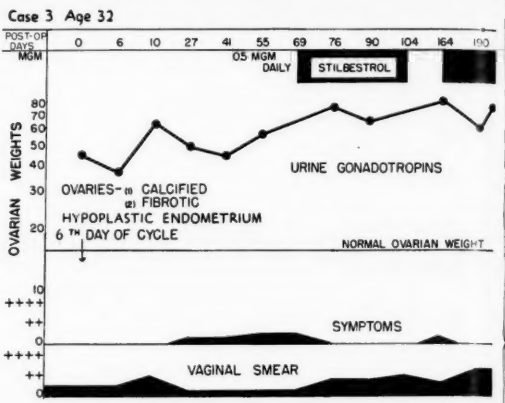

FIG. 2.

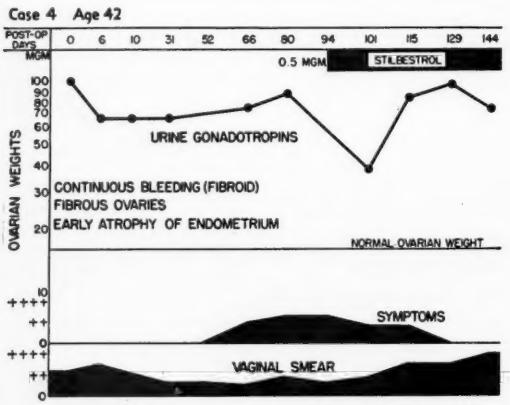

FIG. 3.

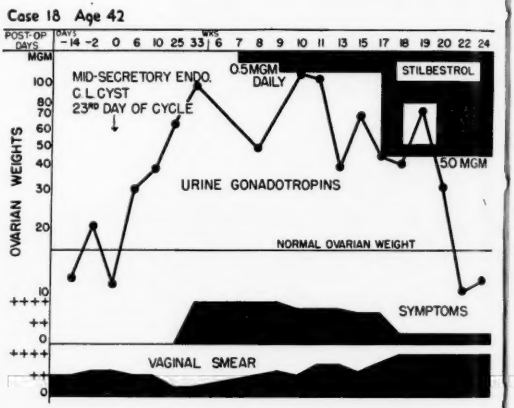

FIG. 4.

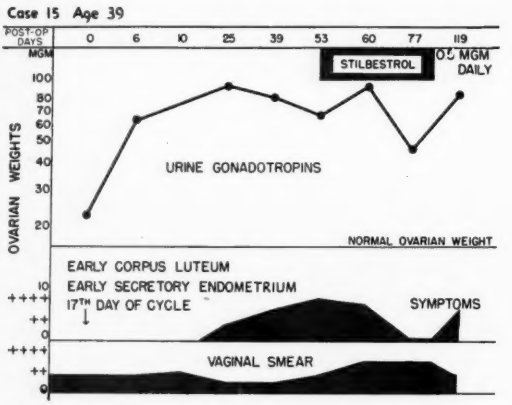

FIG. 5.

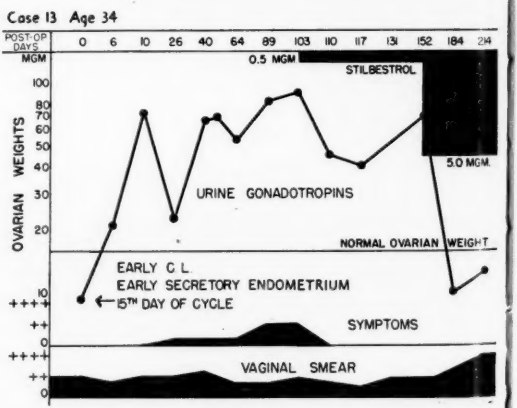

FIG. 6.

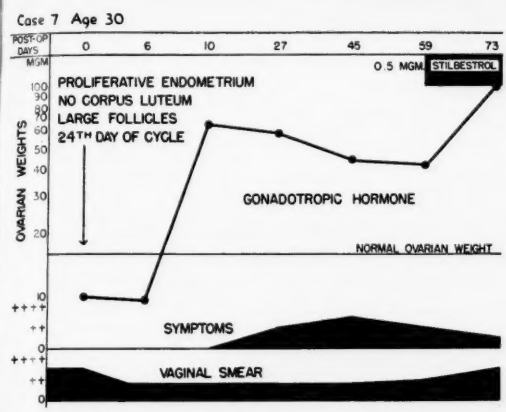

FIG. 7.

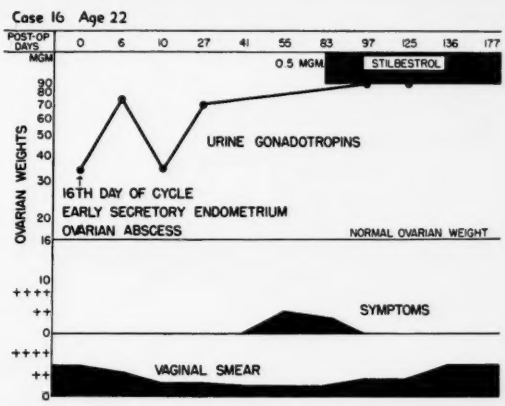

FIG. 8.

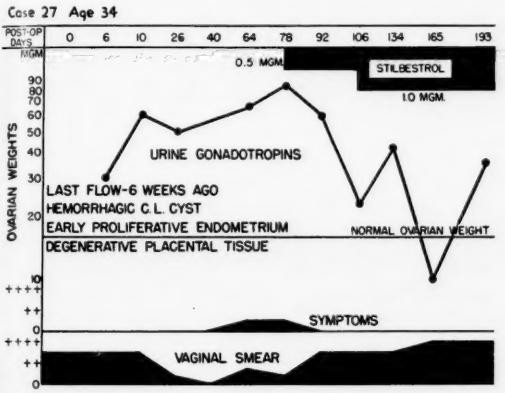

FIG. 9.

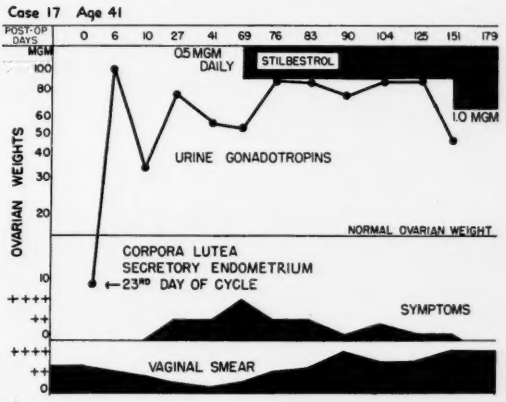

FIG. 10.

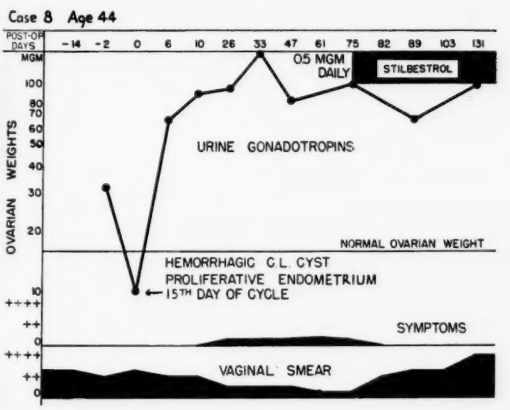

FIG. 11.

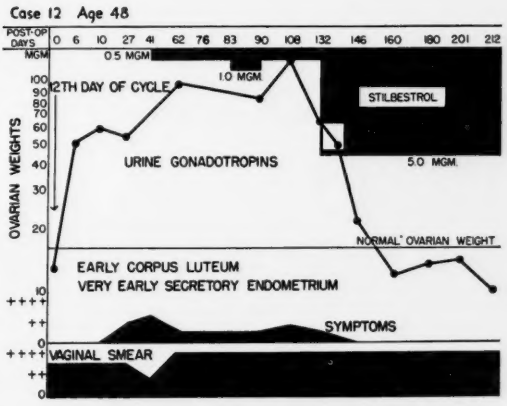

FIG. 12.

were graded as two plus, whereas an average period of 33 days was required for recession from grade two to one plus.

Complete retrogression to smears graded zero. Complete atrophy of the vaginal smear was observed in a total of eight cases. This included four of the five cases in whom atrophic ovaries and uteri were found at operation (*Cases 3* and *4;* fig. 2, 3) and included all three cases with cystic ovaries (*Case 27*, fig. 9). Thus complete retrogression of vaginal cytology occurred in only a minority of cases within 60 to 90 days after castration. In the majority, the post-castrational changes in the vaginal smear were not striking, confirming the findings of McLaren (2).

GONADOTROPHINS

Rapidity in rise. The most striking feature of this study was the rapidity in the rise of urinary gonadotrophins following bilateral oöphorectomy. Of 14 cases who had "low" titers pre-operatively, 13 were restudied on the sixth post-operative day. The urinary gonadotrophins had reached the menopausal zone by the sixth day in six cases (*17, 8, 12;* fig. 10, 11, 12 respectively) and had shown a definite rise in 4 others (*18, 13;* fig. 4, 6 respectively). By the tenth post-operative day, the titer was "high" in eleven of the fourteen cases and was definitely above the pre-operative level in the other three cases.[4] These three cases had titers in the menopausal zone at the next assay conducted at the end of the fourth week. Of seven cases who had "peak" titers pre-operatively, six were restudied on the sixth post-operative day. The urinary gonadotrophins had reached the menopausal zone by the sixth day in five cases (*15, 16;* fig. 5, 8) and by the tenth day in all seven cases. Thus in the combined group of 21 cases with low or peak gonadotrophin excretion pre-operatively, titers in the menopausal zone were obtained in 58 per cent of those studied on the sixth post-operative day; in 86 per cent by the tenth day; and in 100 per cent, by the end of one month.

Maintenance of menopausal levels. After the titer rose to the menopausal level, it remained in this zone as long as treatment was withheld. This observation held true in all 27 patients, including *Cases 1–5* who had high initial titers associated with atrophic ovaries and *Case 27* whose pre-operative level was not obtained.

Control cases. The following cases served as operated controls: one patient having a simple bilateral salpingectomy, one having resection of corpus luteum cysts, and two having unilateral oöphorectomies performed. Menses occurred at regular intervals following operation in all. Gonadotrophins exhibited normal cyclic changes, vaginal smears remained normal, and no menopausal symptoms developed.

DISCUSSION

Correlation of pre-operative status with post-castrational changes.

Cases with atrophic ovaries. The development of definite menopausal symptoms by four of the five cases with atrophic ovaries deserves comment, particularly in view of the fact that two of the group had passed through a spontaneous menopause some years prior to the operation (*Cases 1* and *2*). It is difficult positively to exclude suggestion as a factor in the symptoms reported, even though special precautions were taken to avoid suggestion during the course of each interview. However, we believe that the symptoms recorded were real and not imaginary. The development of symptoms was unrelated to gonadotrophin output since one case had a higher titer pre-operatively than when symptoms were present; two cases maintained an almost constant plateau whereas only one case had a higher titer at the time symptoms were present than before operation. The appearance of menopausal symptoms after the removal of ovaries which were judged to be functionless on the basis of ovarian and uterine cytology would suggest that these atrophic ovaries were still secreting minimal amounts of estrogen. This is further supported by the disappearance of symptoms after small doses of stilbestrol. These observations indicate that much less ovarian function is required to prevent menopausal symptoms than to maintain normal uterine and vaginal epithelium and to keep gonadotrophins at normal levels. We

[4] In *Case 25* this rise was indicated by marked uterine stimulation and canalization of the vagina of the assay rat.

therefore suggest that atrophic ovaries be left *in situ* in order that their minimal secretion may prevent or lessen menopausal symptoms.

Cases with functional ovaries. No correlation was demonstrable between the stage of the ovarian cycle at the time of oöphorectomy and 1) the rapidity or severity of the menopausal symptoms, 2) the degree of retrogression of the vaginal smear, or 3) the rapidity and height of the rise of urinary gonadotrophins.

Correlation of menopausal symptoms with post-castrational changes in vaginal smear.

Retrogression of the vaginal cytology antedated the appearance of symptoms in twelve cases (*4, 7, 16, 17, 27;* fig. 3, 7, 8, 9, 10); took place simultaneously in two cases (*8;* fig. 11); and occurred subsequently to the establishment of symptoms in one case (*Case 12*, fig. 12). Moreover, the degree of retrogression in the vaginal smear was unrelated to the severity of the symptoms. Six patients had severe symptoms with little or no change in vaginal smear (*15, 18;* fig. 4, 5) whereas two had marked retrogression in vaginal cytology with minimal symptoms (*Cases 8, 27;* fig. 9, 11).

Correlation of gonadotrophic titers with menopausal symptoms.

The first rise in post-castrational levels of gonadotrophic hormone occurred in all 22 cases with normal or cystic ovaries before any symptoms had developed. In the five cases with atrophic ovaries the level was already high before symptoms were encountered. This is a clear indication that menopausal symptoms (including hot flashes) are not immediately due to the rise in gonadotrophins. All gonadotrophin levels reached the menopausal zone promptly. Within this zone variations in recipient rat ovarian response could not be correlated with severity of menopausal symptoms.

Correlation of gonadotrophins with vaginal smear values.

The rise in gonadotrophins to the menopausal zone was so uniform and prompt, whereas the vaginal smear response was so variable, that no correlations could be established.

Diagnostic inferences. Since gonadotrophins were always elevated promptly following castration, one could predict that they would be elevated in: (1) castrated women with menopausal symptoms; (2) castrated women whose vaginal smear values were zero; and (3) in any women one month after castration. One could not predict that menopausal symptoms would be present, or smear values would be low from the finding of elevated gonadotrophins. The vaginal smear was the most capricious and unreliable index of the physiological and clinical status of the post-castrational women.

Elevation of gonadotrophins, fall in vaginal smear value and occurrence of menopausal symptoms are all the result of oöphorectomy. No one of these is dependent upon either of the other two, however. The study confirms the impression that menopausal symptoms are due to estrogen withdrawal and are not due to gonadotrophin rise.

SUMMARY

Twenty-seven bilaterally oöphorectomized women were observed at regular intervals for development of changes in urinary gonadotrophins, vaginal smear values and menopausal symptoms from the time of operation until post-castrational changes were firmly established.

The first and most striking change occurred in the urinary gonadotrophic titers. All with normal pre-operative levels showed a significant rise in gonadotrophin excretion as early as the sixth to tenth post-operative day. The menopausal zone was reached by the sixth day in 58 per cent, by the tenth day in 86 per cent and by the next assay conducted at the end of one month in all cases studied. Five patients with pre-operative levels in the menopausal zone maintained these levels after castration.

Menopausal symptoms made their appearance and attained their maximal severity between the twenty-seventh and sixty-sixth post-castrational day in the average patient. The most common symptoms were hot flashes, nervousness and sweating. Loss of libido was common.

Retrogression of vaginal cytology occurred in most cases, but could not be correlated with

either the rise in gonadotrophins or with development of symptoms.

Four of five patients whose pre-operative gonadotrophic titers were high and whose ovaries, uteri and vaginal mucosa were atrophic responded to castration by developing menopausal symptoms. This suggests that atrophic ovaries may still be secreting minimal amounts of estrogen and should be preserved at operation if possible.

REFERENCES

1. HELLER, C. G., J. P. FARNEY, D. N. MORGAN AND G. B. MYERS: A correlation of the ovarian and endometrial histology, vaginal epithelium, gonadotrophic hormonal excretion and the day of the menstrual cycle in 28 women. *J. Clin. Endocrinol.* 4: 95. 1944.
2. MCLAREN, H. C.: The normal menopause; the induced menopause. *J. Obst. & Gynec. Brit. Emp.* 48: 1. 1941.

Effect of Small and Large Doses of Diethylstilbestrol upon Menopausal Symptoms, Vaginal Smear and Urinary Gonadotrophins in 23 Oöphorectomized Women[1,2]

CARL G. HELLER,[3] M.D., Ph.D.,
ROBERT E. CHANDLER, B.S., AND
GORDON B. MYERS, M.D.

From the Department of Medicine, Wayne University College of Medicine, and the Medical Service of Receiving Hospital, Detroit, Michigan

THE efficacy of diethylstilbestrol in treatment of the menopause has been universally accepted, but disagreement still exists as to the mode of action of both synthetic and naturally occurring estrogens. Albright (1), Frank and Salmon (2), Jones, MacGregor and Tod (3), Rowlands and Sharpey-Schafer (4), Lawrence and Moulyn (5) and others have implied that pituitary suppression was necessary for relief of menopausal symptoms since the estrogen doses which they used did reduce the urinary gonadotrophin output. On the other hand Heller and Heller (6) and Shorr (7) succeeded in abolishing the symptoms associated with the physiological menopause by small doses of the naturally occurring estrogens which did not cause a measurable depression of urinary gonadotrophic output. In our opinion the divergent conclusions are explainable by the differences in the type of estrogen, the dosage and the route and frequency of administration employed by the various clinics.

Since these investigations were conducted, many clinics have adopted the use of orally administered diethylstilbestrol in the treatment of the menopause. This synthetic estrogen has provided the opportunity of making comparisons between clinics since it is of uniform purity and potency and can be given in weighed amounts. It has generally been found that a dose of 0.5 mgm. daily by the oral route will produce satisfactory results in the average patient. The effect of relatively small and relatively large oral doses (0.5 mg. and 5.0 mg. daily respectively) upon menopausal symptoms, vaginal smears and urinary gonadotrophins was accordingly determined in women who had been subjected to bilateral oöphorectomy (8) and followed postoperatively until the menopausal syndrome had been firmly established (9).

PROCEDURE

The test subjects were 23 patients who had had bilateral salpingo-oöphorectomy as well as hysterectomy. They all showed elevated gonadotrophins, castrational vaginal smears and menopausal symptoms (8). Methods used in determining gonadotrophins, in grading vaginal smears and in evaluating menopausal symptoms have been described in the companion papers (8, 9). The sole therapeutic agent

[1] Aided by a grant from Frederick Stearns and Company, administered by Dr. Richard M. Johnson, Medical Director.

[2] Presented in part before the 26th Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, New Jersey, June 8, 1942.

[3] Research Fellow, American College of Physicians, 1942–43.

was diethylstilbestrol[4] and this was invariably administered by mouth. Placebos were tried in a few cases, but sedatives, analgesics, etc., were not used. The initial dose for the entire group of 23 patients was 0.5 mg. daily. Treatment was started between the fortieth and sixtieth and urinary gonadotrophic output. These studies were performed seven days after the beginning of treatment in 14 cases, on the fourteenth day in 18 cases, on the twenty-first or twenty-eighth day in 17 cases, between the thirty-fifth and forty-ninth day in 13,

TABLE 1. RESPONSE TO 0.5 MG. OF DIETHYLSTILBESTROL, ORALLY ADMINISTERED, DAILY

Case No.	Post-op. Day Rx Was Initiated	Symptoms				Vaginal Smears				Urinary Gonadotrophins			
										Pre-Rx Averages[4]		During Rx Averages	
		Pre-Rx	Rx Full Effect	Rx Time of 1st Effect	Rx Time of Full Effect	Pre-Rx	Rx Full Effect	Rx Time of 1st Effect	Rx Time of Full Effect	No. of Assays	Recipient Rat Ovarian Weight	No. of Assays	Recipient Rat Ovarian Weight
		Grade[1,3]	*Grade*	*Days*	*Days*	*Grade*[2,3]	*Grade*	*Days*	*Days*		*Mg.*		*Mg.*
1	83	0.5	0	7	28	0	3	7	63	7	74	3	93
2	83	2	0	7	30	0	4	7	57	5	83	4	108
3	69	1	0	7	21	0	2	7	36	6	49	5	69
4	94	1.5	0	7	36	1	4	7	55	6	81	4	75
6	69	2	0	7	21	1	3	7	7	6	50	3	57
7	59	2	1	14	—	2	3	14	14	4	52	1	131
8	75	0.5	0	7	7	0	2	7	14	6	99	2	88
9	53	2.5	0	14	42	1	4	14	91	4	58	4	62
10	40	3	0	14	28	—	4	—	28	1	62	3	72
12	41	2.5	1	21	21	2	4	21	21	3	54	3	94
13	103	1.5	0	7	7	1	2	28	28	7	66	3	50
14	59	3	0	14	28	2	2.5	14	14	4	48	3	54
15	53	4	0.5	7	24	2	3	7	7	7	77	3	87
16	83	1.5	0	14	14	1	3	14	53	3	60	2	91
17	69	4	0.5	7	56	1	3	7	21	5	65	6	92
18	49	4	4	—	14	2	2.5	7	14	2	79	1	49[5]
20	60	2	0	7	28	2	4	7	14	4	68	6	89
21	139	4	0	7	21	2	2.5	7	7	2	66	3	81
22	84	1	0	7	7	3	4	7	7	2	60	1	50[6]
24	83	4	0	14	28	1	3	14	14	6	68	2	67
25	80	2	0.5	32	32	0	2	7	7	4	60	3	78
26	91	2	0	14	14	0	2	7	7	2	55	1	75
27	78	1	0	14	14	1	3	14	14	4	64	1	60
									Total	100	Ave. 66.02	67	Ave. 78.6

Time of any reported change is dated to coincide with the day patient reported the change; thus changes may have occurred considerably earlier, depending upon how long the delay between interviews.

[1] Grade of symptoms is indicated: 0 for none; 0.5 to 1.0 for mild; 1.0, 1.5 and 2.0 for moderate; and 2.5 to 4.0 for severe.

[2] Grading of vaginal smears is discussed in a previous paper (10).

[3] Pre-treatment grades recorded for symptoms and vaginal smears were the last observations made before therapy was instituted.

[4] Pre-treatment averages for urine gonadotrophin assays were taken from the time the assays entered the menopausal zone.

[5] This drop in titer is more apparent than real, for on the 1 mgm. dose 5 assays averaged 75 mg. See chart *Case 18*.

[6] Depressed assay.

postoperative days in seven patients, between the sixtieth and eightieth postoperative days in six, between the eightieth and one hundredth days in eight, and after the one hundredth day in the remaining two patients. Following institution of therapy, frequent observations were made of menopausal symptoms, vaginal smears between the fifty-sixth and seventy-seventh day in 11, between the seventy-seventh and ninety-eighth in 7, and on the one-hundred-nineteenth day after therapy was initiated in 2 cases. The response to a dose of 1.0 mg. by mouth daily was similarly determined in six of the group and, finally, the effect of a daily oral dose of 5.0 mg. was determined in a total of 10 cases. The significant data are summarized in tables 1 and 2. The case numbers correspond

[4] The diethylstilbestrol was kindly contributed by Dr. Richard M. Johnson, Medical Director, Frederick Stearns and Co., Detroit.

to those of table 1 in each of the two companion articles (8, 9). The graphic illustrations of some of the cases are included in the second paper.

Results from 0.5 Mgm. Diethylstilbestrol Daily

Effect upon symptoms. Demonstrable improvement of the menopausal symptoms occurred in all cases except *Case 18* (fig. 4)[5] who was observed for only two weeks on this dosage scale. Twelve patients noticed distinct improvement within seven days after the institution of therapy, as illustrated by *Cases 6, 13, 17, 27* (fig. 13, 6, 10, 9); eight more reported alleviation of symptoms at the interview on the fourteenth day; and the other two had partial relief at the end of twenty-first and thirty-second days respectively.

Complete abolition of all menopausal symptoms occurred in 17 cases on the 0.5 mgm. dose. Three additional cases obtained almost complete symptomatic relief (*Cases 15, 17, 25*—fig. 5, 10, 14). Two continued to have definite but mild (one plus) symptoms (*Cases 7* and *12*—fig. 7 and 12). *Case 7* did not report after the fourteenth day so it is not known whether more protracted therapy would have been effective. The length of time required for the abolition of symptoms was seven days in three cases, 14 days in three cases, 21 days in three cases, 28 days in five cases, and between 29 and 42 days in three cases. Thus diethylstilbestrol orally in doses of 0.5 mg. daily afforded complete or almost complete symptomatic relief in twenty (87 per cent) of 23 castrate women including seven of nine cases whose symptoms were classed as severe. Placebos, administered for 14 and 40 days in two cases, caused no change in symptomatology.

Eleven women were questioned regarding libido before castration and nine reported normal findings. Of these, six experienced a post-castrational loss of libido and five regained it on the 0.5 mgm. dose level. A sixth patient, who had had no libido pre-operatively, reported the establishment of libido while being treated with 0.5 mgm. diethylstilbestrol. Of the two remaining cases with absent libido, one responded to the administration of 5.0 mgm. of diethylstilbestrol and one did not. This variability of response to castration and to estrogenic therapy suggests that libido is dependent upon both hormonal and psychogenic factors and that either one or the other may play a dominant rôle in its loss.

Effect upon the vaginal smear. The vaginal smears responded to the 0.5 mgm. dose of diethylstilbestrol by increases in activity in all 23 cases, as is evident in the graphs. The first effect of treatment was usually noted on the day of the first interview after institution of therapy. In 14 cases the increased vaginal smear value was demonstrable on the seventh day, in six on the fourteenth day, in one on the

TABLE 2. RESPONSE OF URINARY GONADOTROPHINS[1] TO 5.0 MGM. DIETHYLSTILBESTROL, ORALLY, DAILY

Case No.	Post-castration Day Rx Begun	Average Ovarian Weight During 0.5 Mg. Rx	Days after 5.0 Mgm. Dose Begun								
			7	14	21	28	35	49	60	70	80
		Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
2	182	108				(−)15					
6	132	57					(+)24	(+)27	(+)39	(−)14.3	
9	144	62	(+)85								
10	124	72					(−)13.2				
12	132	94	(+)48	(+)21			(−)12.2	(−)13.9		(−)14	(−)8.2
13	152	50					(+) 9.8			(−)11.8	
18	116	75[2]	(+)41	(+)73	(+)32		(−)10		(−)12		
20	171	89			(−)16						
22	175	50		(+)16.9							
24	216	67			(−)12	(−)15					

[1] Figures given are for recipient rat ovarian weights (normal uninjected controls=8−16.2). (+) indicates concomitant uterine weight stimulation. (−) indicates no uterine stimulation.
[2] On 1.0 mgm. dose.

[5] Figure numbers 1–12 refer to preceding article in this issue.

twenty-first day, and in one on the twenty-eighth day. The remaining case, in whom no pre-treatment grade was determined, had a maximal (four plus) response on the twenty-eighth day. The full effect had been established in 13 cases between the seventh and fourteenth days, in five cases between the twenty-first and thirty-sixth days, and in the remaining five cases between the fifty-third and ninety-first days. The grade of the vaginal smears at the time the full effect was established varied from two plus to four plus. Five cases were graded as two plus, three as two and one-half plus, eight as three plus and seven as four plus. The speed and eventual grade of the smear response could not be related to the pre-treatment grade.

Thus, in contrast to the slow and variable response of vaginal smears to castration, there was an almost immediate, invariable, and usually marked response of vaginal smears to the 0.5 mgm. dosage of oral diethylstilbestrol therapy.

Effect upon gonadotrophins. Prior to the institution of treatment, the urinary gonadotrophic excretion was firmly established in the menopausal zone in all 23 cases as shown by a total of 100 separate urine assays performed during this period. The number of pre-treatment assays for each patient is indicated in table 1, together with the average gonadotrophic output, recorded in terms of recipient rat ovarian weight. During the administration of 0.5 mg. diethylstilbestrol daily, a total of 67 separate urine assays were performed on the 23 patients and are recorded in table 1 in the same fashion as the pre-treatment assays. The average recipient rat ovarian weight for the 100 pre-treatment assays was 66.02 mgm. During treatment with 0.5 mg. diethylstilbestrol suppression of urinary gonadotrophins did not take place. In fact, the average for the 67 assays performed during the administration of diethylstilbestrol gave a weight of 78.6 mgm., which was actually higher than the average before treatment.

Doses of 0.5 mg. diethylstilbestrol daily did not depress the gonadotrophic titer to levels below menopausal zone in any case. Slight decreases in titer were noted during treatment in six cases, but were not attributable to the diethylstilbestrol in at least two cases (*18* and *22*). In *Case 18*, a single assay during the administration of 0.5 mg. was below the pre-treatment level but an average of five assays during the administration of 1.0 mg. was almost identical with the pre-treatment average. The assay in *Case 22* was known to be too low due to a laboratory mishap. On the other hand, 17 patients exhibited rises in urinary gonadotrophins during the administration of 0.5 mg. diethylstilbestrol daily. The highest levels were generally obtained in the assays made after one week of treatment. Some astonishingly large rat ovaries were occasionally encountered at this time.

The presence of diffuse luteinization of the follicles of recipient rat ovaries or the formation of corpora lutea was occasionally noted in the larger ovaries encountered in assays of urine from patients prior to initiation of therapy. Corpora lutea and diffuse luteinization were observed with greater frequency after treatment was begun.

In the pre-treatment assays the more common uterine finding was ballooning with fluid, indicating estrogenic activity, whereas in the assays performed during treatment it was more common to find thick-walled non-translucent uteri containing no fluid. The latter type of uteri indicate progesterone stimulation, which in turn suggests that during treatment greater amounts of interstitial-cell-stimulating hormone were present.

Response to 1.0 Mgm. of Diethylstilbestrol

In six cases, 1.0 mgm. of diethylstilbestrol, by mouth, daily was substituted for the 0.5 mgm. dose. Only three of these cases had menopausal symptoms. *Case 12* (fig. 12) obtained no relief from symptoms of grade 1 severity. The very mild symptoms of *Case 17* were abolished, while the severe symptoms of *Case 18* (fig. 4) were reduced from grade 4 to grade 3. The vaginal smear values rose to the maximum of estrogenic activity (grade 4) in all cases except *Case 18*, in which they rose to grade 3. Gonadotrophic assays were performed in three cases. In *Case 1* (fig. 15) they remained in the menopausal zone when tested at the end of 14 days; in *Case 18* the average of five assays, determined during 45 days of treatment, remained at 75 mgm., which was the pre-treatment level; and in *Case 27*, (fig. 9), during 87 days of therapy, gonadotrophin assays dropped

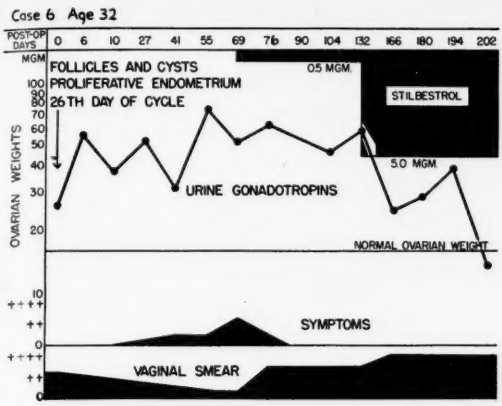

FIG. 13.

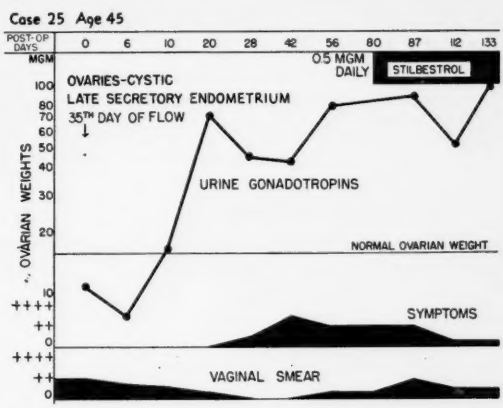

FIG. 14.

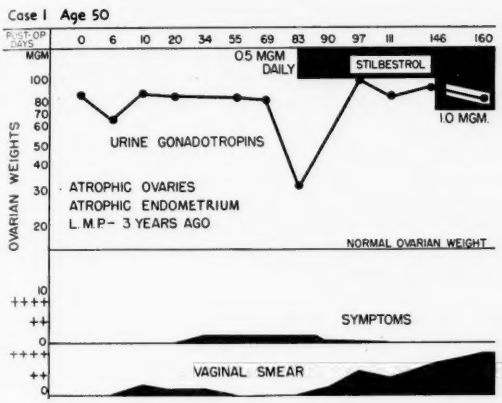

FIG. 15.

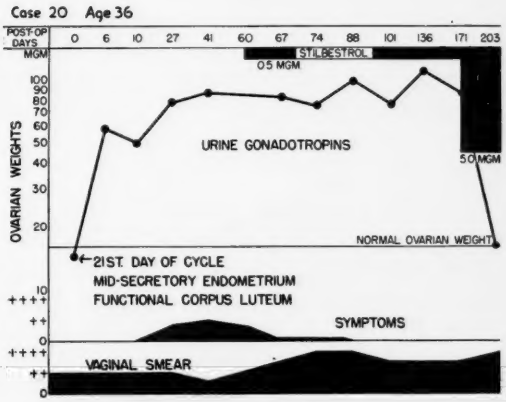

FIG. 16.

to 39 mgm. as compared with a pre-treatment average of 64 mg.

Response to 5.0 Mgm. of Diethylstilbestrol

In ten cases 5.0 mgm. of diethylstilbestrol given by mouth each day was substituted for the previous 0.5 mgm. or 1.0 mgm. dose. The menopausal symptoms of only two of the ten cases had not responded to the lower doses. In *Case 12* (fig. 12) residual grade 1 symptoms were immediately abolished, and in *Case 18* (fig. 4) grade 3 symptoms were reduced to grade 1. All vaginal smears responded by showing maximal, four plus activity, except *Case 9*, who remained under observation only one week.

Urinary gonadotrophins (table 2) fell below the menopausal zone as early as 14 days after the dose of 5.0 mg. was instituted. Each assay performed on or after 21 days in each patient tested indicated levels below the menopausal zone. Eight of the ten cases showed complete absence of any detectable gonadotrophic hormone in the urine as illustrated by *Cases 6, 12, 13, 18, 20* (fig. 13, 12, 6, 4, 16). *Case 9*, studied only one week, showed no drop; and *Case 22*, studied once at the end of two weeks, showed depression into the normal zone. The fall in gonadotrophins in the eight cases having completely negative assays indicates a drop of hormone below that excreted by normal women.

DISCUSSION

Origin of menopausal symptoms. A controversy has existed as to the relationship of the pituitary to menopausal symptoms. Albright (1), Nathanson, Rice and Meigs (10), Lawrence

and Moulyn (5), Fluhman (11), and Kurzrok and Smith (12) have implied that high gonadotrophins are responsible for the hot flashes and other symptoms observed in the menopause, whereas we are certain they are not due to elevated gonadotrophins and believe they are due to estrogen withdrawal. Our studies of bilateral oöphorectomy have shown that: 1) gonadotrophic titers are in the menopausal zone for at least two weeks and as long as seven weeks before the onset of menopausal symptoms; 2) the titers at the time symptoms appeared were on the average no higher than in the two to seven week interval when symptoms were absent; 3) there was no correlation between height of the titer and severity of the symptoms; 4) when symptoms were alleviated by administering small doses of diethylstilbestrol the gonadotrophic titers not only did not fall, but tended to increase.

While the above evidence in our opinion is conclusive, it is by no means the only evidence bearing on this subject. Other relevant data include the following.

1) All women who enter the menopause have elevated urinary gonadotrophins (6), yet many of these women fail to develop climacteric symptoms.

2) Women in the post-menopausal period continue to have elevated gonadotrophins at the same high levels as women with menopausal symptoms, although they remain asymptomatic (6).

3) Women with menopausal symptoms obtain relief from the administration of the naturally occurring estrogens without concommitant depression of urinary gonadotrophins (6).

4) Prepuberal gonadal failure associated with high urinary output of gonadotrophic hormone is not characterized by climacteric symptoms in either males or females. No climacteric symptoms were encountered in the women with primary amenorrhea, short stature and high gonadotrophins described by Varney, Kenyon and Koch (13) and Albright, Smith and Fraser (14). None was encountered in males classed as functional prepuberal castrates by Heller, Nelson and Roth (15). No menopausal symptoms occur in males suffering from the syndrome described by Klinefelter, Reifenstein and Albright (16) and Heller and Nelson (17) in which high gonadotrophins and normal 17-ketosteroid values co-exist.

5) Climacteric symptoms have been observed by us in three cases in which gonadotrophin excretion was below normal. The symptoms were due to failure of the gonads secondary to pituitary failure. Two of these subjects, one male and one female, died of Simmonds' cachexia. At autopsy the male was found to have a chromophobe adenoma (18) and the female had atrophy of the anterior lobe of the pituitary. The climacteric symptoms in the two male cases were alleviated by administration of chorionic gonadotrophins.

The foregoing evidence conclusively indicates that elevated gonadotrophins are not responsible for menopausal symptoms. It is probable that estrogen withdrawal causes symptoms because: 1) Evidence of ovarian failure is invariably present; 2) symptoms are promptly and specifically relieved by estrogenic therapy. The marked variation in severity of symptoms noted in the present and other studies suggests that there are other conditioning factors. These include: a) the possibility that atrophic ovaries elaborate minimal amounts of estrogen as exemplified by the appearance of definite symptoms after the removal of atrophic ovaries in *Cases 1* and *2* who had undergone the natural menopause several years beforehand; b) the possibility that estrogen is elaborated in other sites, probably the adrenal glands, as shown by the recovery of estrogens from the urines of castrated cases; c) the possibility that the previous level of circulating estrogens may condition the vasomotor system of women differentially; d) the possibility of psychogenic differences.

Mode of relief of menopausal symptoms. Albright (1), Lawrence and Moulyn (5), Kurzrok and Smith (12), Frank and Salmon (2) and others have implied that gonadotrophic hormone suppression is necessary for abolition of menopausal symptoms; however, this study has shown that gonadotrophic hormone suppression is *not* necessary for symptomatic relief. A daily dose of 0.5 mgm. of diethylstilbestrol alleviated menopausal symptoms in 20 of 23 cases and had an estrogenic effect upon the vaginal mucosa in all cases. Yet this dosage, which exerted both a demonstrable clinical and physiological effect, did not cause a detectable

suppression of urinary gonadotrophins. This clearly indicates that the clinical and physiological effectiveness of the 0.5 mgm. dose of diethylstilbestrol was not mediated through the anterior pituitary gland. These observations confirm earlier studies (6) made on the physiological menopause, in which naturally occurring estrogens were used to alleviate menopausal symptoms. Under these circumstances, likewise, no suppression of urinary gonadotrophins was demonstrable. Results of animal experimentation have also shown that estrogens (19, 20) and androgens (21) may restore various end-organs to normal in castrated animals without causing pituitary gonadotrophic depression. Thus the symptomatic and physiological effects of small but adequate doses of estrogens are independent of the pituitary gland. The mode of relief of menopausal symptoms is rather the correction of an abrupt estrogen deficiency.

Effect of estrogens upon the pituitary. Small doses of estrogen, in the castrate, tend to stimulate rather than depress gonadotrophic excretion, as shown by a slight rise in titer during the administration of 0.5 mgm. of diethylstilbestrol. The rise in titer may be largely due to increases in the luteinizing fraction (interstitial-cell-stimulating hormone). This is suggested by the finding of greater frequency of diffuse ovarian luteinization of the assay rat ovaries and the occurrence of progestational uterine changes in the assay rats.

Definite gonadotrophic suppression can be obtained with daily doses of 5.0 mg. of diethylstilbestrol which are approximately ten times the usual amount required for symptomatic relief and restoration of normal vaginal cytology. In eight cases who received these large doses for four weeks or longer, assays failed to demonstrate the presence of gonadotrophic hormone in the urine.

The use of inordinately large doses of estrogens by both clinical and laboratory workers has led to the erroneous conception that pituitary suppression is necessary for (or always accompanies) reversal of castrational changes.

As shown in animal experiments (19, 21), each end-organ has a different threshold of response to estrogens and androgens. Such differences in threshold are well illustrated by this study of castrated women; menopausal symptoms, vaginal smear changes and changes in urinary gonadotrophins did not occur simultaneously after castration or respond similarly to the same dosage of diethylstilbestrol. Satisfactory clinical results can generally be obtained by small doses of estrogens, which do not suppress the gonadotrophic output. The very large doses, which do suppress urinary gonadotrophins, are rarely needed for clinical relief and should ordinarily be avoided because of the excessive stimulation of the uterine and vaginal mucosa and other organs which might eventually be a factor in neoplastic change.

Cause of post-castration rise in gonadotrophins. The evidence in support of the concept that the increased gonadotrophic excretion is not due to increased hypophyseal production but rather to failure of ovarian utilization of the hormone has been recently discussed (19). The data in this study do not vitiate this viewpoint. The prompt rise in gonadotrophins following castration and the subsequent sustained plateau is explained, according to this concept, by failure to utilize gonadotrophins as a result of oöphorectomy. Further data which directly support this concept have been obtained by transplantation of ovaries to the spleen and will be reported in a separate communication.

Summary of pituitary-ovarian relationship. 1) Gonadotrophic hormone is utilized by normal ovaries but not by castrates or ovaries with impaired function, as a consequence of which much larger amounts are available for excretion. 2) Estrogens administered in small amounts increase the output of the luteinizing hormones. It is probable that peaks of estrogen production by the normal ovary have a similar effect. 3) The fluctuations in gonadotrophin excretion during the normal menstrual cycle may be due to either variations in the amount of luteinizing hormone produced or to variations in the amount of gonadotrophin utilized by the ovaries. 4) Large doses of estrogens administered to castrates have the opposite effect from small doses, suppressing or completely inhibiting gonadotrophic output. The suppression by large doses may represent an exhaustion of secretory granules of the pituitary cells as suggested by A. E. Severinghaus (22). It is possible that long-continued, smaller doses may have a like effect. Administered es-

trogens may have similar effects in normal females.

SUMMARY

The effect of relatively small and relatively large oral doses of diethylstilbestrol was determined upon menopausal symptoms, vaginal smears and urinary gonadotrophins in 23 women who had been subjected to bilateral oöphorectomy and followed post-operatively until the menopausal syndrome had been firmly established.

Daily doses of 0.5 mgm. by mouth alleviated menopausal symptoms in 20 cases and had an estrogenic effect upon the vaginal mucosa in all 23 cases but did not suppress gonadotrophic excretion. The average titer of gonadotrophins during the administration of this dosage was slightly higher than before treatment due to increased output of the luteinizing fraction (interstitial-cell-stimulating hormone). The symptomatic and physiological effects of the 0.5 mgm. dose of diethylstilbestrol were not mediated through the anterior pituitary gland.

Daily doses of 5.0 mgm. of diethylstilbestrol administered for one month or longer in eight cases caused complete suppression of gonadotrophic output.

An explanation is offered for the divergent opinions concerning the mechanism of production of hot flashes and other menopausal symptoms and the mechanism of relief by estrogenic therapy.

REFERENCES

1. ALBRIGHT, F.: Studies on ovarian dysfunction. III. The menopause. *Endocrinol.* 20: 24. 1936.
2. FRANK, R. T., AND U. J. SALMON: Effect of administration of estrogenic factor upon hypophyseal hyperactivity in the menopause. *Proc. Soc. Exper. Biol. & Med.* 33: 311. 1935.
3. JONES, M. S., T. N. MACGREGOR AND H. TOD: Oestradiol benzoate therapy in depressions at the menopause. *Lancet* 1: 320. 1937.
4. ROWLANDS, I. W., AND E. P. SHARPEY-SCHAFER: Effect of oestradiol benzoate on the amount of gonadotrophin in pituitary gland and urine after the menopause. *Brit. M. J.* 1: 205. 1940.
5. LAWRENCE, C. H., AND A. C. MOULYN: The menopause: A hormonic and therapeutic study. *New England J. Med.* 224: 845. 1941.
6. HELLER, C. G., AND E. J. HELLER: Gonadotrophic hormone: Urine assays of normally cycling, menopausal, castrated, and estrin treated human females. *J. Clin. Investigation* 18: 171. 1939.
7. SHORR, E.: The menopause. *Bull. New York Acad. Med.* 16: 453. 1940.
8. HELLER, C. G., J. P. FARNEY, D. N. MORGAN AND G. B. MYERS: A correlation of the ovarian and endometrial histology, vaginal epithelium, gonadotrophic hormonal excretion and the day of the menstrual cycle in 29 women. *J. Clin. Endocrinol.* 4: 95. 1944.
9. HELLER, C. G., J. P. FARNEY AND G. B. MYERS: The development and correlation of menopausal symptoms, vaginal smear and urinary gonadotrophin changes following castration in 27 women. 1944. *J. Clin. Endocrinol.* 4: 101. 1944.
10. NATHANSON, I. T., C. RICE AND J. V. MEIGS: Hormonal studies in artificial menopause produced by roentgen rays. *Am. J. Obst. & Gynec.* 40: 936. 1940.
11. FLUHMAN. C. F., AND K. M. MURPHY: Estrogenic and gonadotrophic hormones in the blood of climacteric women and castrates. *Am. J. Obst. & Gynec.* 38: 778. 1939.
12. KURZROK, R., AND P. E. SMITH: The menopause. A. *Research Nerv. and Ment. Dis., Proc.* 17: 340. 1938.
13. VARNEY, R. F., A. T. KENYON AND F. C. KOCH: An association of short stature, retarded sexual development and high urinary gonadotrophin titers in women. *J. Clin. Endocrinol.* 2: 137. 1942.
14. ALBRIGHT, F., P. H. SMITH AND R. FRASER: A syndrome chracterized by primary ovarian insufficiency and decreased stature. Report of 11 cases with a digression on hormonal control of axillary and pubic hair. *Am. J. M. Sc.* 204: 625. 1942.
15. HELLER, C. G., W. O. NELSON AND A. A. ROTH: Functional prepuberal castration in males. *J. Clin. Endocrinol.* 3: 573. 1943.
16. KLINEFELTER, H. F., JR., E. C. REIFENSTEIN, JR. AND F. ALBRIGHT: Syndrome characterized by gynecomastia, aspermatogenesis without aleydigism and increased excretion of follicle-stimulating hormone. *J. Clin. Endocrinol.* 2: 615. 1942.
17. HELLER, C. G., AND W. O. NELSON: Hyalinization of seminiferous tubules associated with elevated gonadotrophins in males with or without aleydigism, gynecomastia and eunuchoidism. To be published.
18. FOSTER, M. A., AND J. C. MCCARTER: Simmonds' disease associated with pernicious anemia, with bioassay of large chromophobe adenoma. *J. Clin. Endocrinol.* 1: 436. 1941.
19. HELLER, C. G., E. J. HELLER AND E. L. SEVRINGHAUS: Does estrogen substitution materially inhibit pituitary gonadotropic potency? *Endocrinol.* 30: 309. 1942.
20. LAUSON, H. D., C. G. HELLER AND E. L. SEVRINGHAUS: Inadequacies of estradiol substitution in ovariectomized albino rats. *Endocrinol.* 23: 479. 1938.
21. HELLER, C. G., A. SEGALOFF AND W. O. NELSON: Effect of testosterone propionate on the pituitary gonadotropic potency of the castrated male rat. *Endocrinol.* 33: 186. 1943.
22. SEVERINGHAUS, A. E.: The cytology of the pituitary gland. *A. Research Nerv. and Ment. Dis., Proc.* 17: 69. 1938.

Effects of Massive Doses of Testosterone Propionate upon Spermatogenesis

ROBERT S. HOTCHKISS, LT. COMDR. (MC) U.S.N.R.[1]

San Diego, California

THE RESULTS of the administration of androgens upon the germinal epithelium are still controversial (1). This is due, in part, to the variations in response by the different species which have been used as subjects for tests. Experiments on the rat, squirrel, bird, monkey, and its use in man have produced such conflicting results that it is apparent conclusions derived from observations on one species may be erroneous for another [Moore (2), Moore and Price (3), Wells and Moore (4), Wells (5), Cuyler (6), Heckel (7, 8), McCullagh and McGurl (9), Korenchevsky and Hall (10) and Korenchevsky et al. (11)]. Furthermore, at least three additional fundamental considerations must be borne in mind in evaluating the effects of androgens upon the testes: namely that the response of testicular tissue is dependent upon (1) the state of the other internal glands, (2) the condition of the germinal epithelium prior to treatment, and (3) the dosage and period of time over which the androgens are given.

Experiments upon animals with unbalanced endocrine systems are well known and have yielded basic information concerning the interrelationship of the endocrines. Hypophysectomized animals have been protected against the usual post-operative testicular atrophy by being supplied with androgens [Walsh et al. (12), Nelson (13)]. The combination of adrenal cortical hormones and androgens has been shown by Nelson to be effective in restoring spermatogenesis weeks after the removal of the pituitary gland.

[1] This case study was completed before entering the service of the United States Navy. The opinions and assertions contained herein are the private ones of the writer and are not construed as official or reflecting the views of the Navy Department or the naval service at large.

Clinical studies of sterile men have disclosed great variations in the states of the germinal epithelium. Testicular biopsies have repeatedly demonstrated that advanced atrophy of the germinal epithelium is possible in testes of normal size and consistency. It is obvious that such testes are incapable of responding to any known form of treatment and hence it is of prime importance to determine the state of spermatogenesis before instituting any type of therapy.

The amounts of androgens used and the time interval over which they are given apparently determine whether spermatogenesis will be depressed or stimulated. Moore (2) has shown that in rats repeated small injections were more harmful than administration of larger amounts. Shay et al. (14) and Rubinstein and Kurland (15) likewise reported that stimulation or inhibition of spermatogenesis was dependent upon the amounts of androgens given. Heckel (7, 8) and McCullagh (9) have demonstrated in man that repeated injections of testosterone propionate over a period of weeks caused profound but transient depression of spermatogenic activity.

The following study is presented with the foregoing factors in mind.

CASE REPORT

Past history and examination. An exceptionally well-developed, 30-year-old man was first examined on October 9, 1941. At the age of five he had mumps which did not cause pain or enlargement of either testicle. No other illnesses were known. He had been married for four years and had used contraceptives for the first two years of marriage. For the past two years, however, coitus had been practiced without contraceptives twice a week, but his wife had failed to conceive. Physical examination disclosed no abnormalities. Secondary masculine traits were well developed and

there were no suggestions of an endocrine disorder. The external genitalia were normal in appearance. Both testes were of normal size and were in the proper location, but the consistency of each was somewhat soft. The prostate and seminal vesicles were not remarkable. The laboratory findings were essentially normal except for the semen analysis which revealed the following findings.

FIG. 1. Pre-medication testicular biopsy.

FIG. 2. Post-medication testicular biopsy.

Volume	7.0 cc.
Viscosity	reduced
Turbidity	reduced
Motility	1 sluggishly motile sperm per 20–30 HPF. 1 immotile spermatozoön per 5–6 HPF
Cell count	Too few cells for accurate count

Three subsequent specimens of semen were collected at weekly intervals, preceded by at least two days of sexual abstinence. Analysis of these showed no material change. Unquestionably there was marked oligospermia and subnormal motility.

On November 15, 1941, testicular biopsy specimens were secured (fig. 1). Each testis exhibited considerable tubular fibrosis. Spermatogenesis seemed to be almost completely arrested at the stage of the secondary spermatocyte and only an occasional mature spermatozoön could be found. An unfavorable prognosis was given the patient who nevertheless requested that any possible corrective measures be tried no matter how remote the possibility of improving his fertility.

TREATMENT

(a) *Chorionic and pituitary gonadotropins.*[2] Alternate intramuscular injections of chorionic gonadotropic and pituitary gonadotropic hormones were given four times a week for ten weeks beginning December 30, 1941. The amounts thus administered totaled 10,000 I.U. of the former and 1000 R.U. of the latter. This medication was supplemented by oral ingestion of 50 mg. of nicotinamide a day. Following the last injection, the semen was collected and examined once a week for eleven weeks. No material changes were noted and it was concluded that the treatment had been ineffectual.

(b) *Testosterone propionate.*[2] A plan was devised to administer, within a short period of

[2] The author is deeply indebted to Roche-Organon, Inc., of Nutley, New Jersey, for their supplies of endocrine preparations used in this study. Pregnyl, Ambinon and Neo-Hombreol (the latter in a special concentration not commercially available) were the products employed.

time, relatively large amounts of this androgen. A highly concentrated preparation containing 50 mg. of testosterone propionate per cc. in oil solution was made up for the purpose.

From June 8, 1942 through June 11, 1942, a dose of 250 mg. of testosterone propionate was injected intramuscularly daily for four days—a total of 1000 mg. The semen was analyzed each week for the following four months. Considerable variation in cell counts was observed. The best specimen during this observation period was that obtained on August 10, 1942. The total cell count was 3,960,000 with 1 to 7 moderately active cells seen per HPF (high power field).

From October 13, 1942 through October 18, 1942, the patient was given a second intensive course of treatment, 300 mg. of testosterone propionate being injected daily for five days—a total of 1500 mg. Repeated semen analyses during the subsequent nine weeks again disclosed marked variability in the cellular contents of the semen and in the motility of the cells. The specimen examined on December 28, 1942 was found to be the best during this period and revealed the following findings.

Volume	8.4 cc.
Count per cc.	1,200,000
Total Count	10,080,000
Motility	3–4 moderately active cells per HPF. Motility was maintained for eight hours

From December 28, 1942 through December 31, 1942, the patient was given a third series of intramuscular injections of testosterone propionate, totaling 3000 mg. Sperm counts made during the next three months ranged between 3,825,000 and 5,400,000 spermatozoa in each specimen. The motility was always definitely subnormal.

From March 15, 1943 to April 13, 1943, 4500 mg. of testosterone propionate was given as follows: 100 mg. on each of 11 consecutive days and 200 mg. on each of the subsequent 17 days. A semen specimen examined on April 15, 1943 was completely devoid of spermatozoa. Repeated checks were made every five days and no spermatozoa were found in the semen until May 3, 1943 when one immotile sperm was observed in a centrifuged specimen. Examinations were continued at less frequent intervals during the next six months. A gradual improvement was observed and two months following this last course of treatment the total counts averaged between one and two million spermatozoa. One to four sluggish sperm cells were seen in each HPF.

On May 10, 1943, a biopsy of the right testis was taken again (fig. 2) and compared with the pre-medication specimen. The germinal epithelium exhibited the same type of tubular fibrosis and low degree of spermatogenesis. There was no evidence of change in spermatogenic activity nor indication that the previous treatment had altered the general structure of the testis.

DISCUSSION

A young man with faulty spermatogenesis of unknown etiology was first treated with chorionic and pituitary gonadotropins. No response was observed despite the fact that the germinal epithelium had exhibited certain degrees of spermatogenic activity and that a few mature spermatozoa had been found in the semen.

Then the patient was subjected to intensive androgenic treatment. A total of 10,000 mg. of testosterone propionate was administered in four courses extending over a ten-months' period according to the following plan:

	Total
250 mg. each on 4 consecutive days (June, 1942)	1000 mg.
300 mg. each on 5 consecutive days (October, 1942)	1500 mg.
600 mg., 600 mg., 1000 mg., and 800 mg. respectively on 4 consecutive days (December, 1942)	3000 mg.
100 mg. on 11 consecutive days followed by 200 mg. on 17 consecutive days (March–April, 1943)	4500 mg.

Each course of treatment was followed by long observation to detect any possible latent effects of the medication.

As is shown by the above, a total of 5500 mg. of testosterone propionate was given to the patient during the first three courses. The single doses were as high as 250 to 1000 mg.; this is in contrast to the lower individual doses—100 and 200 mg. respectively—administered during

the fourth course of treatment. Repeated semen analyses disclosed one significant difference: the large doses of testosterone propionate given during the first three courses did not inhibit spermatogenesis and spermatozoa were found in each instance. In contrast, the 28 days of consecutive treatment with smaller doses produced definite inhibition of spermatogenesis as indicated by the absence of spermatozoa in the semen during a period of 18 days following the fourth course of treatment. However, the azoöspermia was transient and spontaneous recovery ensued. During the first 14 days of this course, the patient noted a period of increased libido. He also reported a gain of seven pounds and observed a slight edema of the face. These signs and symptoms disappeared at the conclusion of the 28 days of continuous treatment.

Frequent semen analyses made following the courses with high individual doses of testosterone propionate disclosed variation in the number of sperm cells present in the various specimens. The activity of the cells was inconstant but remained consistently subnormal. There were no changes of sufficient significance to indicate that spermatogenesis had been stimulated. The ranges in cell count and in activity of the spermatozoa were compatible with physiologic variations commonly found when repeated examinations of the semen from the same individual are made over a long period of time.

A post-medication testicular biopsy carried out about one month after completion of the fourth course of testosterone propionate failed to disclose any appreciable alteration in the structure of the testis as compared to that prior to treatment.

SUMMARY AND CONCLUSIONS

Treatment of a man with dysfunction of the germinal epithelium of both testes with chorionic and pituitary gonadotropins failed to stimulate spermatogenesis.

Massive doses of testosterone propionate given intramuscularly in several courses of brief duration failed to stimulate or depress spermatogenesis.

Smaller doses of testosterone propionate administered to the same patient by daily injections over a period of 28 days produced a temporary suspension of spermatogenesis.

Analyses of semen, which were made repeatedly, and testicular biopsies were methods used to interpret the effects of testosterone propionate medication on the germinal epithelium. No changes were produced in the structure of the testicular tissue as evidenced by the pre-medication and post-medication biopsy specimens which are presented. No harmful effect on the germinal epithelium could thus be detected following the administration of a total of 10,000 mg. testosterone propionate administered in four courses extending over a period of ten months.

REFERENCES

1. Editorial: *J. Clin. Endocrinol.* 3: 375. 1943.
2. Moore, C. R.: Glandular Physiology and Therapy. Chapt. 16. Am. Med. Assn., Chicago. 1942.
3. Moore, C. R., and D. Price: *Anat. Rec.* 71: 59. 1938.
4. Wells, L. J., and C. R. Moore: *Anat. Rec.* 66: 181. 1936.
5. Wells, L. J.: *Endocrinol.* 32: 455. 1943.
6. Cuyler, W. K.: *J. Clin. Endocrinol.* 3: 375. 1943.
7. Heckel, N. J.: *Proc. Soc. Exper. Biol. & Med.* 40: 658. 1939.
8. Heckel, N. J.: *J. Urol.* 43: 286. 1940.
9. McCullagh, E. P., and F. J. McGurl: *J. Urol.* 42: 1265. 1939.
10. Korenchevsky, V., and K. Hall: *Brit. M. J.* 1: 4. 1939.
11. Korenchevsky, V., M. Dennison and K. Hall: *Biochem. J.* 31: 1434. 1937.
12. Walsh, E. L., W. K. Cuyler and D. R. McCullagh: *Am. J. Physiol.* 107: 508. 1934.
13. Nelson, W. O.: *Am. J. Physiol.* 129: 430. 1940.
14. Shay, H., J. Gershon-Cohen, K. E. Paschkis and S. S. Fels: *Endocrinol.* 28: 485. 1941.
15. Rubinstein, H. S., and A. A. Kurland: *Endocrinol.* 28: 495. 1941.

Treatment of Breast Cancer with Testosterone Propionate

A Preliminary Report

ERIC FELS, M.D.

Cordoba 838
Buenos Aires, Argentina

IN THIS preliminary report no attempt will be made to explain the relationship between sex hormones and cancer in general, the purpose being merely to describe the results obtained with testosterone propionate in three cases of mammary cancer.

Among the few reports published on this use of the male sex hormone, the earliest is that of Ulrich (1) who reported the effects in two cases. The first patient, aged 45 years, had had a unilateral mastectomy (left breast) for intracanalicular epithelioma. The right breast was painful, granular and rough to palpation and seemed to be in a preneoplastic or epitheliomatous state. Since malignant transformation such as had occurred in the left breast was feared, amputation of the right breast was contemplated, but before carrying out this procedure Ulrich gave two series of 10 injections of 10 mg. of testosterone acetate. After the first few injections there was a distinct lessening of pain in the right breast and left arm and by the time the treatment was completed the breast had become supple, almost soft, and was no longer of a nodular consistency. The second patient, aged 42 years, had a massive infiltrating neoplasm of the right breast, palpable tumors in the left breast and a large uterine fibroma. When first found the neoplasm of the right breast was completely inoperable. A hysterectomy with removal of the tubes and ovaries was performed and eight days later a double series of injections of 5 ampules of 40 mg. each of testosterone acetate was administered. Four weeks later a similar series was given. Immediately following the beginning of injections pain diminished and five weeks after the operation the neoplasm had decreased in size, had become mobile and was operable.

Loeser (2, 3) reports the case of a 37-year-old patient who had undergone a radical mastectomy for cancer of the breast, with subsequent x-ray therapy. Eight months later there was a recurrence below the right clavicle. A curettage, performed two months later because of uterine hemorrhage, revealed endometrial hyperproliferation. The uterine bleeding was treated by administration of a total of 1500 mg. of testosterone propionate during the next 5 months. This resulted in amenorrhea. Another 600 mg. of testosterone propionate was injected after an interval of three months and thereafter 600 mg. of testosterone propionate was implanted at intervals of 3, 6 and 6 months respectively. A year and a half after the beginning of androgen therapy there had been no recurrences. Another case of Loeser's, treated by radical mastectomy and x-ray therapy for breast cancer, suffered about six months later a recurrence in the scar, which was excised. Nine months later there was another recurrence in the supraclavicular region. A curettage, performed because of uterine hemorrhage, revealed a hyperplastic endometrium. From April, 1937 to July, 1938 a total of 2800 mg. of testosterone was given. Up to one and one-half years after the institution of androgen treatment no further recurrences had taken place.

Loeser considers it particularly noteworthy that there were no recurrences after the treatment with testosterone propionate in spite of the fact that, without testosterone therapy, cancer had recurred very early.

Of the four cases mentioned, only Loeser's

second case seems to prove that the hormone treatment brought about the favorable effect. Loeser's first case was treated simultaneously with x-ray therapy and testosterone propionate which makes it impossible to decide which measure was responsible for the inhibition of recurrences of new foci of cancer. Ulrich cautions that one case does not necessarily prove a point but states that "it is perhaps permitted to us to hope that, thanks to testosterone hormone therapy, we have at our disposal perhaps not only a powerful medication for combating chronic mastitis, but in addition for preventing the appearance of a certain number of benign tumors arising from mastitis."

Farrow and Woodard (4) treated 33 patients with bone metastases from breast cancer with doses of 5 to 25 mg. of testosterone propionate one to three times a week for 10 to 12 doses and observed a decrease in pain in about one-half of the patients treated.

CASE REPORTS

Even though our own cases have been observed for a comparatively short time, a preliminary report on our experiments to date appeared to be warranted.

Case 1. Age 34 years, Czech, married. Menstruation occurred every four weeks and lasted for 2 to 3 days. On first examination, October, 1941, she had a uterine fibroma reaching almost to the umbilicus. In the right breast there was a tumor, the size of a small apple, slightly fixed to the skin. Biopsy revealed a carcinoma of the alveolar type (fig. 1).

A roentgenographic examination made on October 27, 1941, by Dr. Schlanger, showed infiltration of subacute character in the right pulmonary hilum. Visible parts of the skeleton were without lesions.

Radical mastectomy was performed on November 3, 1941. Histologic examination revealed that some of the axillary glands were invaded by the epithelioma (fig. 2).

Roentgen-ray treatment was given prophylactically. The general condition was good until March, 1942 when the patient started to complain of pains in the sacrum and in the region of the lower left ribs. The pains increased rapidly and the patient became bedridden. In April, intermittent vomiting started which sometimes lasted entire days and interfered with proper nutrition. The condition of the patient became constantly worse. The pains could be somewhat alleviated only by morphine and chloral hydrate.

A roentgenographic examination, made on July 27, 1942 by Dr. Schlanger, showed the classic appearance of generalized metastases, characterized by general decalcification of the skeleton and multiple, round, destructive lesions in the lower left ribs, in the thoracolumbo-sacral column, and in the pelvic bone. No bone neoplasm was visible anywhere. The disease progressed and the treatment was purely symptomatic.

A roentgenographic examination made on November 6, 1942, by Dr. Schlanger, showed that the bone lesions had progressed in extent and size. Areas most attacked were the hips, the pelvis, and the entire spine including the thoracic and cervical parts. The lesion of the right pulmonary hilum had improved. Menstruation had not occurred since March, 1942. In March, 1943 a gibbosity appeared on the thoracic column and at the same time hard nodules were noticed in the right supraclavicular region. These grew slowly until they formed large clusters. The pains were always very intense, more so sometimes in the region of the hips, sometimes in the pelvis, the thighs, or in the lumbar or cervical region, so that the patient could not move her head.

The daily dose of sedatives had reached 0.15 to 0.2 gm. of morphine and two ampules of pantopon. Even so the pain was nearly unbearable. The vomiting persisted and increased the degree of malnutrition so that the patient was cachectic. The pulse was sometimes very small, necessitating the use of cardiac stimulants.

With the patient in this desperate condition, treatment with male sex hormone was started on July 24, 1943, in the form of testosterone propionate,[1] one 25-mg. ampule every other day. After the administration of 250 mg. of testosterone propionate, vomiting diminished and eventually disappeared entirely. Toward the end of August there was also a marked improvement with regard to the "neuralgic" pains and there was an increase in appetite. Four weeks later, after more than 700 mg. of testosterone propionate had been administered, the fibroma had disappeared; the uterus was normal in shape and size; and the nodules in the supraclavicular and cervical region had decreased in volume. After having been bedridden for 18 months the patient left her bed for the first time. The only side effects noted were: intense pain in the lower abdomen and the sensation of an impending menstruation (which, however, did not occur) after the administration of 250 mg. of testosterone propionate and signs of virilization after administration of 700 mg.

A roentgenographic examination made on September 24, 1943, by Dr. Schlanger, showed that the bone lesions had not changed in extent; and toward the end of October the gibbosity had nearly disappeared. Examination of the blood showed no abnormalities. The patient took one-hour to two-hour airings a day and the daily doses of morphine could be reduced to 0.08 to 0.10 gm. On November 1, 1943, the total amount of androgen injected had reached 1100 mg. and the signs of virilization had become more and more evident. Sometimes there occurred colic pains in the lower abdomen and very slight vomiting at intervals of 10 or 15 days, apparently in connection with high outdoor temperature and humidity.

[1] The androgen used was Neo-Hombreol (Organon).

In place of the large nodules in the supraclavicular region previously mentioned, only a small hard nodule the size of an apple pip was found. In the muscles of the right cervical region some rigidity existed. At this time the patient felt so well that she played ping-pong daily.

On December 3, 1943, the small nodule in the cervical region was excised under local anesthesia. Microscopic examination revealed nests and strings of carcinomatous cells, apparently intact. However, fibrous tissue strewn with epithelial elements predominated (fig. 3, 4).

Comparison of this biopsy tissue with that taken almost two years before (fig. 2) revealed that the changes in the ratio between the neoplastic epithelial tissue and the fibrous tissue were decidedly in favor of the latter.

A roentgenographic examination made on December 20, 1943, by Dr. Schlanger, showed a definite improvement in the appearance of the skeleton. The general calcification of the skeleton had increased; the lacunar lesions had decreased in size and some had partly disappeared; and in some vertebrae hyperostosis was noticeable. The most evident changes were

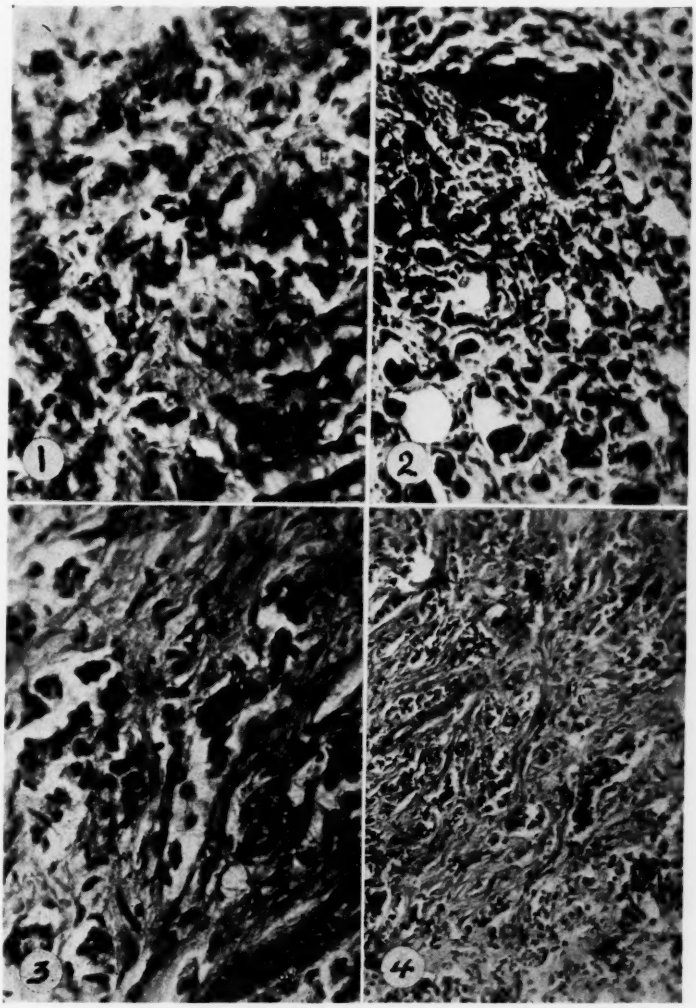

FIG. 1. Histological section of mamma. *Case 1.*
FIG. 2. Axillary lymph node invaded by metastatic neoplasm. *Case 1.*
FIG. 3, 4. High and low power photomicrographs of a cervical lymph node showing metastasis. *Case 1.*

observed in the pelvic bones, the pubis, the ischium and in the entire spine.

Administration of androgen was discontinued after a total of 1550 mg. had been reached and up to the time of writing the condition of the patient has remained unchanged.

Case 2. Age 48 years, German, married. The patient, who had undergone radical mastectomy of the right breast for cancer two years ago, was first seen at the end of October, 1943 in a very bad condition. She had been confined to bed for 7 months with intensive vomiting and migrating pains and exhibited a serious cachexia and marked anemia; hemoglobin was 50 (Sahli); R.B.C. 2,950,000; leucocytes, 6800; and cytologic findings were normal.

A roentgenographic examination made on November 2, 1943, by Dr. Schlanger, showed generalized advanced metastases in the entire skeleton (numerous round destructive lesions of large size in the pelvis, the thighs, the entire vertebral column, the clavicles, the ribs, and the skull).

Treatment with testosterone propionate, 25 mg. every other day, was initiated, but after 500 mg. had been administered the gains continued undiminished, the anemia and cachexia were more pronounced and there was difficulty in emptying the bladder. The only improvement consisted in cessation of the vomiting, with consequent improvement in the nutrition of the patient.

When a total dose of 750 mg. had been reached the pain decreased, but this may not have been the result of treatment since even before treatment the intensity of the pain had varied. The continued absence of vomiting, however, could definitely be attributed to the hormone therapy.

Case 3. Age 54 years, English, single. The patient had had a tumor of the left breast for the past 6 years and histologic examination revealed cancer. The patient had refused mastectomy. Treatment had consisted of roentgen-rays and radium, and electrocoagulation because of ulceration of the tumor. When first seen in November, 1943, a deep ulcer the size of the palm of a hand occupied the site of the left breast. The ulcer had irregular edges and was completely fixed to the ribs. It gave off a fetid, purulent secretion and was surrounded by numerous nodules (skin metastases). The secretion was so abundant that it required one to two dressings a day.

The right breast had become a large hard tumor which was also fixed but had not ulcerated to the outside. It represented a typical picture of "cancer en cuirasse." There were skin metastases, but no bone metastases were demonstrated roentgenographically. Blood cell count and cytologic findings were normal.

Although no benefit was expected from hormone therapy in this case, it was undertaken because the brother of the patient, a physician, insisted on a trial of this therapy. All that can be reported at this time is that the secretion of the ulcer has noticeably decreased and that the granulations, which were sloughing before, now exhibit a reddish, clean aspect.

DISCUSSION

No claim is made that a cure of cancer has been found. In the first case, although the most recent biopsy still shows intact nests of neoplastic cells, there is definitely a surprising improvement, almost reaching a clinical cure. The improvement consists of subsidence of pain and vomiting, disappearance of palpable nodules as well as of the gibbosity of the vertebral column and, above all, skeletal changes, as evidenced by roentgen-ray examination. Since none of these phenomena have ever been obtained by any other treatment, this success must be attributed solely to the administration of testosterone propionate. The disappearance of a large uterine fibroma during the hormone therapy must be mentioned as a concomitant though less surprising phenomenon.

In the second case the hormone treatment was started at a very advanced stage, in the presence of extreme cachexia and numerous metastases in the entire skeleton. Nevertheless, the subsidence of vomiting after hormone treatment constituted an indubitable improvement. The effect upon the pain is still questionable.

In the third case, a special form of mammary cancer (*cancer en cuirasse*), the period of observation has been too short to permit definite conclusions. All that can be said is that the clearing up of the large ulcer has progressed and the fetid secretion has diminished noticeably after the administration of testosterone propionate.

What explanation can be given for the improvement obtained after treatment with testosterone propionate, particularly in the first case? The favorable influences of this hormone derivative on the so-called mastopathy are well known, but cancer is a very different problem. However, we have one objective finding on which an opinion may be based, i.e. the biopsy taken in the first case after intensive treatment with testosterone propionate. We notice in the sections a pronounced proliferation of the fibrous tissue which surrounds the carcinomatous nests, while no signs of a lesion from real tumor cells are visible. We therefore assume, with the reservations necessary because of our limited experience, that testoster-

one propionate produces an extraordinary proliferation of the fibrous tissue which impedes and blocks the extension of the neoplastic cells and possibly crushes them; but there are no signs which permit us to believe that the hormone has a direct effect on the carcinomatous cells.

Although it is still too early to arrive at definite conclusions, the favorable therapeutic effect is so obvious and the improvement so surprising that we consider it our duty to publish our findings for the sake of arousing the interest of other investigators in this important field of research. Definite conclusions as to the value of this therapy will be reached as time goes on and as the number of cases increases.

SUMMARY

The favorable results obtained with testosterone propionate in the treatment of three patients with mammary cancer are discussed.

REFERENCES

1. Ulrich, P.: Testostérone (hormone mâle) et son rôle possible dans le traitement de certains cancers du sein. *Acta Union internat. contre cancer* 4: 377. 1939.
2. Loeser, A. A.: Subcutaneous implantation of female and male hormone in tablet form in women. *Brit. M. J.* 1: 479. 1940.
3. ——— Mammary carcinoma, response to implantation of male hormone and progesterone. *Lancet* 2: 698. 1941.
4. Farrow, J. H., and Woodard, H. O.: Influence of androgenic and estrogenic substances on serum calcium. *J. Am. Med. Assn.* 118: 339. 1942.

Experimental Studies of Sexual Behavior in Male Mammals[1]

FRANK A. BEACH, Ph.D.

From the Department of Animal Behavior, American Museum of Natural History, New York, N. Y.

THERE ARE few aspects of human existence of greater importance to the clinician than that of sexual behavior. Yet there is probably no other major sphere of man's daily life so poorly understood and so infrequently and ineptly studied as his sexual behavior. Some of the sexual taboos typical of our culture have been relaxed appreciably during the past two or three decades; but social convention still raises discouraging barriers to the experimental investigation of human sexuality. It is to be earnestly hoped that direct, intensive study of the physiological and psychological bases for sexual arousal and the resultant overt behavior in human beings will become possible in the near future.

Investigations of this character have been conducted with animals other than man, and no physician need be reminded of the great body of currently useful medical knowledge which was originally derived from experimentation with animals. Many recent studies of sexual behavior in lower mammals have yielded data which appear to have a direct bearing upon the understanding of human sexual reactions.

ONTOGENETIC DEVELOPMENT OF MATING BEHAVIOR

The mating performance of males of certain mammalian species appears to be innately organized in reasonably complete form. For example, male rats have been reared in isolation cages from weaning until sexual maturity; and when such animals are placed with a receptive female rat they usually mate as readily and as accurately as an experienced copulator (6, 7, 8, 10, 30).

If immature male rats are observed carefully their prepuberal activities are seen to include a stereotyped series of responses which includes most of the elements of the adult copulatory pattern (author's unpublished results). The adult mating response usually appears at 45 days of age or later; but as early as the 19th to the 21st day the young male begins to exhibit pursuit and mounting of a second animal with occasional palpating movements of the forelimbs during the clasp. This is an incomplete form of the adult pattern; and it occurs weakly and infrequently in the immature animal. However, it is important to note that some prepuberal "sex play" does appear regularly in an animal as low in the mammalian scale as the rat. Although it does occur, practice in the mating act is not essential in this species, for males raised in isolation are able to mate with the female in the absence of experience.

Whereas the mating behavior of the males in certain lower mammalian species appears in well-developed form without opportunity for learning, this is not true in the case of the higher primates. The inexperienced male chimpanzee appears at first unable to copulate with the experienced female even though she may attempt to assist him assume his sexual rôle. Students of chimpanzee behavior agree that experience and practice are essential to the biological adequacy of the male's sexual performance and still more important to perfection and skill (12, 38).

In this connection it is important to note that both male and female chimpanzees in-

Submitted by request.

[1] Experiments reported from the Department of Animal Behavior at The American Museum of Natural History constitute part of a research program which has been generously supported by The Committee for Research on Problems of Sex, National Research Council.

dulge in frequent and highly variable sexual behavior 4 and 5 years before reproductive maturity (12).

INDIVIDUAL DIFFERENCES IN SEXUAL EXCITABILITY

Results of studies of sexual behavior in lower mammals give great emphasis to the magnitude and importance of individual differences in excitability. Research workers and physicians concerned exclusively with human subjects are prone to forget that there are marked physiological and psychological differences among individual members of other mammalian species. The extent of such intra-species variation is readily apparent in the susceptibility to sexual arousal shown by different males of the same genetic strain.

The majority of inexperienced male rats (animals raised in isolation) exhibit sexual excitement and copulatory behavior only when they are exposed to a particular pattern of multisensory stimulation; and this pattern is most completely presented by the receptive female of their own species. There are, however, a few individuals whose threshold of sexual excitability is lower than average and who, therefore, may be aroused by stimulus objects inadequate to effect arousal in most males of their species (8). Such sexually "hyperexcitable" rats may attempt to copulate with non-receptive female rats or with other males. At the opposite extreme of the scale of sexual responsiveness are found a few males (possessed of histologically normal gonads and apparently healthy in every respect) which fail to give evidence of arousal even when placed with an extremely receptive female. Every colony contains a small minority of such "non-copulators" (1–3, 6–10, 20, 25, 35, 37).

There is every reason to believe that males of all other vertebrate species, from fish to chimpanzee, show similar inherited individual differences in susceptibility to sexual arousal. As will become apparent later, the possible effects of such differences figure strongly in any theoretical interpretation of sexual phenomena.

RÔLE OF THE NERVOUS SYSTEM

The sensory receptors. One profitable field of experimentation with the sexual behavior of animals lies in investigations of the rôle of the nervous system. Several attempts have been made to determine the nature of the sensory stimulus responsible for sexual arousal and mating in male mammals. Early experimentalists were handicapped by preconceived notions as to the relative simplicity of such a stimulus. It was often gratuitously assumed that the sexual excitement of lower animals is a simple process, probably dependent upon a single sensory aspect of the estrous female. For example, in many species the odor of the female in heat was presumed to be the one essential stimulus inciting sexual arousal in the male.

Results of recent experimental analyses contradict this type of interpretation. Male rabbits copulate with the receptive doe after surgical procedures have rendered them blind, anosmic, and deaf. In this species many types of sensation appear to contribute to the initiation of sexual excitement of the male but practically any one of them is dispensable as long as others remain (14). In the sexually-inexperienced male rat copulation is possible after removal of the eyes, destruction of the olfactory bulbs, or section of the sensory nerves supplying the vibrissae and the anterior head regions. However, loss of any one of these types of sensation tends to reduce the ease with which sexual arousal occurs and thus to lower the probability of mating behavior (8). In the rat as in the rabbit, sexual excitement occurs in response to the simultaneous activation of several different afferent systems by several different sensory qualities of the receptive female.[2]

Careful analysis of the behavior of inexperienced male rats strongly suggests that the stimulus to which they instinctively respond sexually is a particular pattern of sensations. It is not an odor, a tactile sensation, nor a visual impression which arouses sexual excitement; but rather a given combination of olfactory, tactile, visual and probably other sensations which represent for the rat the sexually exciting stimulus leading to mating behavior. It is worth repeating that in lower mammals recog-

[2] In passing it is well to comment upon the explosion of the long-held hypothesis that sensations arising from distended generative organs, particularly the seminiferous tubules, seminal vesicles and prostate, are the essential source of sexual impulses in the male. Castrates deprived of all accessories exhibit normal libido provided the androgen level is maintained by testosterone administration.

nition of this pattern appears to be innate, as does the execution of the appropriate subsequent behavior.

Sexual arousal and mating behavior in subhuman primates also involves multisensory stimulation. In the chimpanzee tactual and visual receptors are most important during the initiation of copulatory relations. Touch and vision are later supplemented by smell, taste and hearing (13).

The brain. Investigation of various sensory receptors has shown that several exteroceptive systems are normally involved in sexual arousal and mating behavior. Other studies of the nervous system have been directed toward a determination of the rôle of various parts of the brain in reproduction.

In sub-mammalian forms the forebrain is apparently involved in the organization and timing of the various discrete acts which are smoothly concatenated into a serial pattern of courtship and mating. In lower mammals the cerebral cortex seems to contribute little to the motor pattern of copulation. Partially decorticated male rats, *if they copulate at all*, do so as skillfully and as frequently as intact animals. It is safe to conclude that the organization of the copulatory pattern is a function of nervous mechanisms lying below the telencephalon. Neocortical injury is, however, not without effect upon the sexual performance of male rodents. Male rats, sexually active before operation, frequently fail to mate after removal of 20 per cent or more of the cerebral cortex. The exact location of the lesion within the neopallium is unimportant. The significant factor is the amount of tissue removed. The proportion of males ceasing to copulate after operation increases directly with the magnitude of the lesion, until (in our experiments) destruction of 75 per cent of the cortex abolishes mating in all cases.

Although some males may continue to copulate after cortical injury many such animals show an effect of the brain loss. This effect is revealed in a post-operative reduction of the proportion of tests in which mating occurs. Normal males from our colony mate in 80 to 100 per cent of their tests with a receptive female. After loss of 45 per cent of the neocortex some males may continue to copulate; but they tend to do so in only 30 to 40 per cent of the post-operative tests. The probability of mating is appreciably reduced by removal of cortical tissue (6, 7, 16). Some males of relatively low sexual excitability become unresponsive to the estrous female after removal of less than one-half of the total cortical mass. Other individuals with a greater initial capacity for arousal may continue to mate after equally extensive brain injury; but even in such cases arousal becomes more difficult with the result that coition occurs in fewer post-operative tests—presumably those tests during which the female is particularly active and stimulating.

It is fairly certain that the sexually depressing effect of neocortical injury is not due to interference with neuromuscular mechanisms essential to effective copulation. Instead, the effect appears to involve disruption of perceptual functions involved in sexual arousal. It has been stated that the essential stimulus to mating behavior is probably a multisensory pattern. Recognition of a pattern implies perceptual processes which may well be mediated by the cortex. It is quite conceivable that the partially decorticated male fails to experience excitement when confronted by the receptive female because the separate sensory stimuli which she presents are no longer perceptually organized.

Studies of the effects of brain injury on male rabbits suggest that in this species cortical injury exerts a less severe effect than in the male rat (14, 32–34). Completely decorticate male rabbits are reported to continue to mate with the estrous doe. Species differences may be responsible for this variation; but investigational technics employed in the studies of rabbits differed in several important respects from those followed in the work with rats and, therefore, an interpretive comparison of results is rendered extremely difficult.

In the case of the male cat complete removal of the cerebral cortex eliminates mating behavior and destroys completely the various responses ordinarily taken to indicate interest in the female in heat. Decorticate males do not utter the "sex call," and fail to pursue and investigate the estrous queen. Even when she exhibits spontaneous courtship responses—an event which appears to have a strongly exciting

effect upon normal males—the decorticate males show no interest in her (author's unpublished findings).

Restricted cortical destruction may or may not interfere with the male cat's mating performance, depending upon the areas involved. Animals suffering extensive injury to the sensory-motor regions experience great difficulty in making the delicate postural adjustments essential to copulation. Such males nevertheless show no marked diminution in sexual drive and persist indefatigably in their attempts to achieve coition with the receptive female. Ablation of the occipital cortex seriously decreases the male's visually-directed behavior and abolishes his ability to orient properly to the distant female. Cortically blind males emit the sex call frequently and copulate effectively once physical contact with the female has been established.

Experimental results have not yet been analyzed in detail but the essential elements of the picture emerge clearly. The male cat differs from the male rat in the increased extent to which the sensory-motor adjustments involved in mating depend upon the neocortex. The two species are similar in that the capacity for sexual arousal (regardless of the ability to copulate) appears to involve the neopallium as a whole and to be relatively independent of any particular cortical area.

RÔLE OF THE TESTICULAR HORMONES

The importance of the testis to the sexual performance of male animals has been recognized for thousands of years. (Aristotle and undoubtedly others before him noted the reduction of libido in the ox and the gelding.) The fact that it is the testicular hormones which contribute in some manner to the male's capacity for sexual arousal became apparent as soon as the internal secretory function of the gonad was established.

The exact nature of the effect or effects of androgens upon the reproductive proclivities of the male are as yet only partially understood; but certain facts are sufficiently well established to justify attempts at a partial interpretation.

One general type of physiological response to androgens is evidently so far removed from purely sexual behavior that it can be omitted from present consideration. In this category fall such effects as changes in the skeletal system following early testosterone treatment (17). More immediately related to reproductive phenomena are the influences exerted by androgen upon the copulatory organ. It seems clear that the capacity for penile erection is at least in part dependent upon the presence of testicular hormones. Androgenic influences in this regard are facilitative rather than essential (18). Occasional erections occur in castrate animals as well as in congenitally eunuchoid men (19).

The influence of androgens upon various short neuro-motor arcs or reflexes concerned in copulation deserves intensive examination but by far the most striking effect of such hormones is reflected in the increased sexual excitability which they induce.

Action of androgens upon the brain. It is helpful to conceive of sexual excitement as a condition which exists when certain central nervous mechanisms are raised to a given level of activity. It can be postulated that varying degrees of sexual arousal are associated with different intensities of nervous activity within the hypothetical *central excitatory mechanism* (*c.e.m.*). If activity in the *c.e.m.* is low the male exhibits no sexual responses. Somewhat higher *c.e.m.* activity results in active exploration and courtship of the female by the male. When the level of nervous activity within the *c.e.m.* rises to a threshold value, impulses are transmitted to the motor centers by which the copulatory act is mediated and coition occurs.

Individual variations in sexual excitability may well be directly related to inherent differences in the level of activity or the ease of excitation within the *c.e.m.* Certain it is that the responsiveness of the *c.e.m.* is greatly increased by androgens. Testosterone does not organize or alter the form of the motor acts involved in courtship and mating. It does markedly increase susceptibility to sexual excitement. As a working hypothesis it may be assumed that the hormone acts upon the central nervous system in such a way as to lower the threshold of the *c.e.m.* and thus increase this mechanism's excitability.

Immature male animals of many species dis-

play desultory, incomplete mating responses. With the maturation of the testes and the rather sudden increase in androgen secretion the *c.e.m.* becomes highly responsive to nervous impulses arising from certain types of exteroceptive stimulation, sexual excitability rises abruptly and the complete adult pattern appears in vigorous and complete form. The experimentalist can anticipate Nature by supplying the testicular hormone to immature males. When this is done the execution of the adult sexual responses follows promptly. Male rats which have just been weaned (21 days) respond to testosterone propionate by adopting the adult mating pattern which would normally make its appearance some 25 days later[3] (12).

The readiness with which adult mating reactions are evoked in immature, androgen-treated males suggests that the neuromuscular mechanisms responsible for such acts are fully organized long before they are normally activated. The effect of the hormone, whether it is derived from the maturing testis or administered prepuberally by the experimenter, can be interpreted as raising the sensitivity of the *c.e.m.* and thus increasing sexual excitability.

Effects of castration. The psychosexual effects of prepuberal castration and the subsequent administration of androgen are subject to similar interpretation. Adult male animals in which the testes have been removed before puberty usually exhibit a low level of sexual responsiveness characterized by infrequent and incomplete mating activity (pigeon, rat, guinea pig, cat (11, 15, 22, 28, 29)). Presumably such behavior reflects a minimal degree of responsiveness within the *c.e.m.* Injections of testosterone propionate result in the prompt appearance of normal libido and potency. This change in behavior reflects a heightened responsiveness to certain environmental stimulus patterns and results directly from an increased sensitivity within the *c.e.m.*

The results of castration performed after the attainment of sexual maturity differ strikingly from the effects of prepuberal gonadectomy. In those species which have been investigated the vigor and frequency of sexual behavior wanes gradually following loss of the testes in adulthood. In male rats and rabbits complete copulatory responses occasionally occur as long as half a year after operation (35). Since testicular hormones are probably dissipated within a few days after castration (23, 24) the more prolonged survival of sexual responsiveness is best explained on the basis of a relatively enduring change in the nervous system. It may be suggested that once the *c.e.m.* has been "sensitized" by androgen, this neural mechanism remains in an excitable state for some time after the responsible hormones are withdrawn. In the absence of testicular androgens the essential central nervous elements gradually lose their responsiveness. Administration of testosterone quickly restores the reactivity of the *c.e.m.* and evokes the resumption of normal sexual behavior in the adult castrate.

It has been explained that some otherwise normal males appear to be inherently resistant to sexual arousal. Many rat colonies, as mentioned earlier, include a small proportion of males which do not mate and pay very little attention to receptive females. If such animals are treated with testosterone propionate many of them show an increase in sexual responsiveness and proceed to copulate normally (6, 36). Once initiated under the influence of the hormone the tendency to mate often continues after treatment ceases.

Up to this point the function of androgens in awakening and maintaining normal sexual responsiveness has been stressed. Experimentally, it is feasible to demonstrate that, within limits, the maximal degree of potential arousal is directly related to the amount of androgen available. Administration of massive, hyperphysiological doses of testosterone propionate to male rats raises sexual excitability far above normal levels.

The majority of male rats raised in isolation until maturity and then tested with various types of stimulus animals exhibit mating reactions only when confronted with a receptive female of their own species. Males given heavy doses of testosterone propionate for several days before they are tested regularly attempt to mate with non-receptive females, young males, immobilized rats, and even with females

[3] Similar hormone treatment induces crowing, "sex dancing" and treading in male chicks as early as the 15th day after hatching. These responses ordinarily appear in untreated birds during the 5th month (26).

of other species (guinea pig) (9). In this instance the neural mechanisms involved in sexual arousal are so responsive and susceptible to activation that they respond to stimulus patterns which normally would be inadequate to excite them.

Another evidence of the effect of androgen upon sexual excitability is reflected in the revival of mating responses in partially decorticated male rats. Some males which cease to copulate after removal of cortical tissue resume their mating behavior when injected with testosterone propionate (author's unpublished data).

IMPORTANCE OF EXTERNAL STIMULUS CONDITIONS

In considering the importance of various factors in the animal's internal milieu it is easy to lose sight of the controlling influences exerted upon behavior by events in the external environment. Sexual excitement does not arise spontaneously as an accompaniment of critical physiological conditions within the organism. A fairly specific combination of internal events constitutes an essential basis for the generation of arousal; but in some lower animals, at least, the actual occurrence of the sexually excited condition normally appears as a response to stimuli provided by the breeding female.[4]

Under ordinary conditions males of average excitability exhibit sexual arousal and execute mating attempts only when confronted with an estrous female of their own species. The maintenance, intensification, and eventual culmination of this excited condition demands appropriate responses on the part of the female. Males far above average in responsiveness may occasionally display some mating reactions to other males or to non-receptive females; but in the absence of the appropriate reciprocal reactions by the second animal sexual behavior is short-lived and, of course, abortive.

Thus, it is evident that in the active breeding male the arousal and maintenance of sexual excitement is controlled by conditions in the environmental situation. So important are such influences that in special cases they may strongly modify the entire behavior pattern. Sexually inexperienced male rats usually attempt to mate only with the receptive female. In contrast, male rats and rabbits which have been repeatedly tested with receptive females in a particular cage frequently attempt to copulate with almost any other animal encountered in that cage (5, 14, 20). Apparently in such instances the entire test situation has assumed a sexually-exciting character, and the rather specific sensory qualities of the estrous female are no longer essential to the "conditioned" male's arousal. It might fairly be said that in this case the male's inherited sexual responsiveness to a given stimulus pattern has been modified and broadened to include reaction to nonative, learned or acquired patterns.

This "conditionability" of the male's sexual responsiveness bears several highly significant implications. Many phenomena which appear to depend upon or result from this particular capacity of the male are strongly reminiscent of certain aspects of human sexual life. The sexual impulses of man are often said to differ from those of other animals by the intrusion of so-called "psychic" factors. It is frequently implied that the reproductive urge in humans is highly colored and modified by learning, experience, and culturalization. This is undoubtedly true. At the same time it is important to note that the sexual responsiveness of male mammals as far removed from man as the rat is also strongly subject to modification as a result of experience.

Inferential evidence suggests that the modifiability of sexual responsiveness in the male may be due to the fact that under ordinary conditions the cerebral cortex is involved in this form of excitability. It is well known that the acquisition of new habits is mediated in a large part by the cortex. Cortical destruction in the rat interferes with ability to learn a maze (21) and, as has been pointed out earlier, the same type of brain injury tends to depress sexual responsiveness. In this connection it is of interest to note that maze-learning ability and copulatory frequency are positively related in the

[4] The popular and somewhat romantic conception of the rutting male seeking a mate has, so far as I know, little basis in established fact. Males in breeding condition may become more active in their wanderings, often do become more aggressive and pugnacious in their reactions to other adult males; but it is probably incorrect to ascribe their behavior to an impelling "sex urge." It is only when the receptive female is encountered and her peculiar sensory qualities are brought to bear upon the male that his reactions (courtship and mating) can properly be termed sexual.

male rat. Animals which make few errors in learning a maze usually mate with the receptive female in a high percentage of tests; whereas other individuals which make many errors in learning the maze tend to mate in a much lower proportion of their sex tests (5).

BISEXUAL BEHAVIOR

Any discussion of sexual behavior in male animals must include some consideration of so-called "homosexual" responses. The term is highly objectionable, illy defined and so ambiguous that it should have no place in scientific discussion of animal or human behavior. It is usually based upon the sex of the participants rather than the nature of the overt acts performed. In the case of human anal intercourse, for example, the individual performing the masculine role and he who assumes the passive or feminine part are not distinguished—but both acts are indiscriminately labelled "homosexual." Perhaps the strongest indictment against the term lies in the fact that it rests upon wholly gratuitous assumptions regarding the motivation of the individuals so named; it often has little bearing upon the overt behavior involved. We are still embarrassingly ignorant regarding the psychological factors giving rise to the behavior in question (27). Certainly any postulation of a simple and universal type of motivation common to all men and women who engage in sexual relations with individuals of their own sex is unwise and apt to delay the acquisition of valid knowledge of the real facts.

In the case of animals of many species it is quite common to observe one male mounting another and executing the entire copulatory pattern (with the exception, of course, of intromission). Such males, however, quickly shift their attentions to the receptive female if one is available. Their abortive attempts at copulation with other males are most simply interpreted as the result of a high state of sexual excitement occurring when the only possible sexual partner is a second male. It is to be emphasized that, in the rat and probably in many other species as well, behavior of this nature usually occurs only after a male has been initially aroused by direct contact with the estrous female, or when a male is tested under conditions to which he has previously been sexually conditioned (vide supra).

In rare instances male rats which are mounted by other males display many of the reactions characteristic of the estrous female (4, 31). Such animals can scarcely be regarded as exhibiting sex reversal, however, for they mate with and impregnate receptive females. It can, in fact, be demonstrated that the temporary adoption of the feminine behavior pattern is the direct resultant of two factors: the existence of a very high state of sexual excitement and the concomitant application of certain stimuli adapted to the elicitation of feminine responses.

If male rats are treated with large amounts of testosterone propionate they become, as has been explained, highly excitable sexually. Such males readily attempt to mate with various biologically unsuitable incentive animals (9). When these hyper-responsive rats are mounted by other males they often respond by behaving in a manner typical of the receptive female. If an estrous female is then presented, the androgen-treated males shift immediately to the masculine rôle and copulate with her vigorously.

A theoretical explanation of these phenomena may be formulated rather simply. Many if not all males are innately endowed with a neuromuscular organization capable of mediating both the masculine and the feminine copulatory patterns. Both of the neuromotor circuits involved are subject to facilitative impulses arising in the central excitatory mechanism (*c.e.m.*). The threshold of those nervous circuits in which the feminine pattern is organized is quite high in the genetic male; and consequently the feminine response can be elicited only when activity within the *c.e.m.* is intense, in other words, when the male is highly excited. Furthermore, the masculine and the feminine responses are each associated with separate stimulus patterns arising in the external environment. When a male is aroused to a high pitch of sexual excitement he copulates or attempts to copulate with nearly any other animal available, providing the second animal permits such behavior. However, if the stimulus situation does not provide for the release of excitement by the execution of the masculine response; and if at the same time, stimuli nor-

mally associated with the feminine pattern are brought forcibly to bear upon the excited male, he responds by adopting the feminine rôle. The high state of nervous activity within the *c.e.m.* cannot discharge into effector circuits mediating the masculine response and opportunity for discharge in another direction is available; consequently the neuro-motor mechanisms for feminine behavior are activated.

Behavior of this type is most properly termed "bisexual." The potentialities include both feminine and masculine patterns. The directive factors lie in the nature of the external stimulus situation. The behavior pattern which eventually appears will be determined by the stimulus pattern which is brought to bear.

SUMMARY

Experimental investigation of the sexual behavior of male animals has yielded data which suggest the following tentative conclusions.

Males of many if not all mammalian species exhibit some sexual responses well before puberty. These reactions usually consist of incomplete forms of the adult male's mating performance.

Among lower mammals prepuberal practice or experience is not essential to effective copulation when maturity is attained. Male rodents raised in isolation until adulthood and then confronted with a receptive female almost always mate as accurately and as frequently as experienced copulators.

Among higher primates (chimpanzees specifically) the sexually inexperienced male is unable to achieve coition with the receptive female. In this species practice and experience appear to be essential to the efficacy of the male's reproductive behavior.

In all mammalian species which have been carefully studied the male's sexual arousal seems to occur in response to a combination of different types of sensation which are associated with the receptive female. There is little evidence to support the widely accepted belief that a simple sensory stimulus (such as the odor of the female in heat) is the single critical factor giving rise to sexual excitement in the male. The natively adequate stimulus situation appears to be a pattern of multisensory character. Recognition of this pattern is probably innate in lower mammals.

Extensive injury to the cerebral cortex in male rodents does not seriously interfere with the motor acts involved in copulation. Such brain invasion does, however, reduce susceptibility to sexual excitement. Males with large cortical lesions are aroused to the point of copulation with great difficulty if at all; but when mating does occur its accuracy, frequency and vigor are normal. The effects of cortical loss are interpreted as interference with the recognition of the essential stimulus pattern.

Testicular hormones exert a facilitative effect upon susceptibility to sexual arousal in the male. Androgens appear to increase the responsiveness of certain central nervous mechanisms to peripheral excitation. The particular mechanisms most likely to be involved are those concerned with the integration of separate sensory stimuli into a perceptual pattern and not the executive circuits in which the mating pattern is organized.

Although susceptibility to sexual arousal rests upon several critical physiological conditions within the organism (organization of certain nervous mechanisms, level of androgen, etc.), the actual occurrence of an excited state and the resultant overt behavior are strongly influenced by events in the external environment. A male may be in a state of sexual readiness but the arousal of sexual excitement occurs in response to exteroceptive stimuli presented by the receptive female.

Initial sexual arousal in males of lower mammalian species (little is known concerning primates) commonly occurs only in response to a receptive female of the same species. However, responsiveness can be conditioned by experience, and males in which such conditioning has taken place frequently display excitement and attempt to mate with animals other than the receptive female, such as a non-receptive female, other males, or even animals of other species. This modifiability of sexual excitability is thought to be related to the fact that the cerebral cortex is involved in the male's processes of arousal.

Males of some species occasionally exhibit feminine sexual behavior. Such behavior is "bisexual" rather than "homosexual" for the

same animals often exhibit the masculine and the feminine patterns alternately, the deciding factor being the type of stimulus pattern which is provided in the environment.

REFERENCES

1. ANDERSON, E. E.: Consistency of tests of copulatory frequency in the male albino rat. *J. Comp. Psychol.* 21: 477. 1936.
2. ANDERSON, E. E.: Interrelationship of drives in the male albino rat. I. Intercorrelations of measures of drives. *J. Comp. Psychol.* 24: 73. 1936.
3. BALL, J.: A test for measuring sexual excitability in the female rat. *Am. J. Psychol.* 107: 698. 1937.
4. BEACH, F. A.: Sex reversals in the mating pattern of the rat. *J. Genet. Psychol.* 53: 329. 1938.
5. BEACH, F. A.: The neural basis of innate behavior. III. Comparison of learning ability and instinctive behavior in the rat. *J. Comp. Psychol.* 28: 225. 1939.
6. BEACH, F. A.: Effects of cortical lesions upon the copulatory behavior of male rats. *J. Comp. Psychol.* 29: 193. 1940.
7. BEACH, F. A.: Copulatory behavior of male rats raised in isolation and subjected to partial decortication prior to the acquisition of sexual experience. *J. Comp. Psychol.* 31: 457. 1941.
8. BEACH, F. A.: Analysis of the stimuli adequate to elicit mating behavior in the sexually inexperienced male rat. *J. Comp. Psychol.* 33: 163. 1942.
9. BEACH, F. A.: Effects of testosterone propionate upon the copulatory behavior of sexually inexperienced male rats. *J. Comp. Psychol.* 33: 227. 1942.
10. BEACH, F. A.: Comparison of copulatory behavior of male rats raised in isolation, cohabitation, and segregation. *J. Genet. Psychol.* 60: 121. 1942.
11. BEACH, F. A.: Copulatory behavior in prepuberally castrated male rats and its modification by estrogen administration. *Endocrinol.* 31: 679. 1942.
12. BEACH, F. A.: Sexual behavior of prepuberal male and female rats treated with gonadal hormones. *J. Comp. Psychol.* 34: 285. 1942.
13. BINGHAM, H. C.: Sex development in apes. *Comp. Psychol. Monogr.* 5: 1. 1928.
14. BROOKS, C. McC.: The role of the cerebral cortex and of various sense organs in the excitation and execution of mating activity in the rabbit. *Am. J. Physiol.* 120: 544. 1937.
15. CARPENTER, C. R.: Psychobiological studies of social behavior in Aves. I. The effect of complete and incomplete gonadectomy on the primary sexual activity of the male pigeon. *J. Comp. Psychol.* 16: 25. 1933.
16. DAVIS, C.: The effect of ablation of neocortex on mating, maternal behavior and the production of pseudopregnancy in the female rat and of the copulatory activity in the male. *Am. J. Physiol.* 127: 374. 1939.
17. GARDNER, W. U., AND C. A. PFEIFFER: Influence of estrogens and androgens on the skeletal system. *Physiol. Rev.* 23: 139. 1943.
18. HAMILTON, J. B.: Induction of penile erection by male hormone substance. *Endocrinol.* 21: 744. 1937.
19. HAMILTON, J. B.: Demonstrated ability of penile erection in castrated men with markedly low titers of urinary androgens. *Proc. Soc. Exper. Biol. & Med.* 54: 309. 1943.
20. JENKINS, M.: The effect of segregation on the sex behavior of the white rat as measured by the obstruction method. *Genet. Psychol. Monogr.* 3: 457. 1928.
21. LASHLEY, K. S.: Brain Mechanisms and Intelligence. Chicago University Press, Chicago, 1929.
22. MOORE, C. R., AND T. F. GALLAGHER: Seminal vesicle and prostate function as a testis-hormone indicator; the electric ejaculation test. *Am. J. Anat.* 45: 39. 1930.
23. MOORE, C. R., W. HUGHES AND T. F. GALLAGHER: Rat seminal-vesicle cytology as a testis-hormone indicator and the prevention of castration changes by testis-extract injection. *Am. J. Anat.* 45: 109. 1930.
24. MOORE, C. R., D. PRICE AND T. F. GALLAGHER: Rat-prostate cytology as a testis-hormone indicator and the prevention of castration changes by testis-extract injections. *Am. J. Anat.* 45: 71. 1930.
25. NISSEN, H. W.: The effects of gonadectomy, vasotomy, and injections of placental and orchic extract, on the sex behavior of the white rat. *Genet. Psychol. Monogr.* 5: 451. 1929.
26. NOBLE, G. K., AND A. ZITRIN: Induction of mating behavior in male and female chicks following injection of sex hormones. Including notations on body weight and comb growth. *Endocrinol.* 30: 327. 1942.
27. RADO, S.: A critical examination of the concept of bisexuality. *Psychosomatic Med.* 2: 459. 1940.
28. SEWARD, J. P.: Studies on the reproductive activities of the guinea pig. III. The effect of androgenic hormone on sex drive in males and females. *J. Comp. Psychol.* 30: 435. 1940.
29. SOLLENBERGER, R. T., AND J. B. HAMILTON: The effect of testosterone propionate upon the sexual behavior of castrated male guinea pigs. *J. Comp. Psychol.* 28: 81. 1939.
30. STONE, C. P.: Congenital sexual behavior of young male albino rats. *J. Comp. Psychol.* 2: 95. 1922.
31. STONE, C. P.: A note on "feminine" behavior in adult male rats. *Am. J. Physiol.* 88: 39. 1924.
32. STONE, C. P.: The effects of cerebral destruction on the sexual behavior of male rabbits. I. The olfactory bulbs. *Am. J. Physiol.* 71: 430. 1925.
33. STONE, C. P.: The effects of cerebral destruction on the sexual behavior of male rabbits. II. The frontal and parietal regions. *Am. J. Physiol.* 72: 372. 1925.
34. STONE, C. P.: The effects of cerebral destruction on the sexual behavior of male rabbits. III. The frontal, parietal, and occipital regions. *J. Comp. Psychol.* 6: 435. 1926.
35. STONE, C. P.: The retention of copulatory ability in male rats following castration. *J. Comp. Psychol.* 7: 369. 1927.
36. STONE, C. P.: Activation of impotent male rats by injections of testosterone propionate. *J. Comp. Psychol.* 25: 445. 1938.
37. STONE, C. P., M. I. TOMILIN AND R. G. BARKER: A comparative study of sexual drive in adult male rats as measured by direct copulatory tests and by the Columbia Obstruction apparatus. *J. Comp. Psychol.* 19: 215. 1935.
38. YERKES, R. M., AND J. H. ELDER: Oestrus, receptivity, and mating in Chimpanzee. *Comp. Psychol. Monogr.* 13: 1. 1936.

Sex Endocrines in Development and Prepuberal Life[1]

CARL R. MOORE, PH.D.

From the Hull Zoological Laboratory, University of Chicago, Chicago, Illinois

AMONG THE large number of contributions to endocrinology during the past twenty years a respectable portion have dealt with internal secretions of the sex glands and internal secretions in relation to sex problems. Considered from the point of view of a complete life history, or ontogeny, involving fertilization, development, juvenile life, puberty, reproduction and senility, the major portion of attention has been given to problems: a) around the puberal age; b) during the age of active reproduction; and c) to some extent to the period of declining activities, suggested by some to be the age of senility.

It is somewhat striking that one of the most influential contributions in stimulating interest in general sex problems, and especially in the relation of sex hormones to these problems, published more than a quarter-century ago, dealt entirely with the effects of sex hormones during embryonic life; yet since that publication investigations dealing with the question of sex hormones during embryonic development have probably received the least attention. The contribution referred to is that of Professor Frank R. Lillie (1) on the freemartin, or the effects of embryonic sex hormones during development in cattle.

The present discussion will be directed towards an attempt to survey briefly some of the problems of sex hormones in the age period prior to, or leading up to, puberty (embryonic and prepuberal life) and to try to offer some suggestions relative to the results that have been obtained, as well as to give some of my impressions of the meaning of these results. Such a small amount of information is available to us in the nature of clear-cut results, and, because of the dangers in carrying forward results obtained from one species of animal either to apply to another, or to suggest general principles, present day interpretations of these results must in large measure be regarded as tentative, perhaps strongly opinionated.

In a general manner, considering the two periods—*embryonic life* during which the reproductive system is being formed and *post-natal prepuberal life* during which there is a gradual leading up by degrees to a condition in which the organism can actually carry on reproduction—we may state the problem as: Do sex hormones play any rôle during these periods? What other major endocrine glands or secretions may come in for consideration? In a more specific manner we might call attention to some of the problems by such questions as: When do gonads begin to secrete hormones? Is sex gland secretion, or that from any other gland, important during embryonic life? At what period in ontogeny do structures or tissues, that normally respond to hormones in adult life, develop their sensitivity or responsiveness to these secretions? What effects may be produced through treatments of the organism with chemical substances exhibiting properties of the natural hormones? These are but samples and many other phases of interest could receive mention.

To turn attention to the question of hormones during embryonic life let us start with Lillie's most beautiful study on heterosexual

Submitted by request.

[1] Presented by invitation before the Session on Endocrinology, American College of Physicians, Chicago, Illinois, on October 14, 1943. Personal researches have received support from a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.

twin development in cattle. The basic conditions present in the freemartin case are the presence in the uterus of the cow of twin embryos, of different sex, whose embryonic membranes have fused so intimately as to lead to fusion of embryonic blood vessels; these conditions provide for an interchange of blood between the two embryos. The facts observed were first, that sexual abnormalities appear only in a female that has developed while joined by the membranes and blood vessels to a male partner; second, no abnormalities were seen except in the female reproductive tract. In the course of development, the ovary of the conjoined female showed either the absence of, or a degenerating, cortical area and a medulla that exhibited excessive growth and development into a structure characteristic of a sterile testis. The female sex ducts (Mullerian) underwent degenerative changes and were largely removed, whereas the male sex ducts (Wolffian) were retained. As a result of these unusual changes, the female embryo became provided with a well-developed male reproductive system. The interpretation of these findings was based upon hormonal actions. It was suggested that the testis, developing more rapidly than an ovary, secreted testis hormones that were carried through the circulation into the female partner. The testis hormone inhibited—and caused the destruction of—the ovarian cortex which was followed by hypertrophy of the medulla to form a testis. The hormone also inhibited development of the female ducts and stimulated the male set of sex ducts (both sets being present in the early embryo), to develop into a typical male reproductive system.

It was this study that laid the foundation for, and actually introduced, the basic conceptions for the rôle of hormones in determining the character of the reproductive system as embryonic development proceeded; thus was laid the foundation for the conceptions current with the majority of biologists at the present time. To my knowledge Lillie is the first to suggest that hormones play a rôle during embryonic life.

Despite the convincing character of these observations and interpretations and the apparent logic inherent in the explanation of the factors influencing normal development, Lillie recognized the desirability of putting the question to test by experiment. It was at his suggestion that I started about 1918 to attempt to produce experimental freemartin conditions; and having now been engaged more or less continuously on the general problem, using several different approaches, I am still unable to reproduce the freemartin condition.

Basic to the conception of the control of sex differentiation by hormones secreted by developing gonads is the endowment of the hormones with two general properties or functions: 1) the stimulation of parts (sex ducts or other accessory organs of reproduction) characteristic of, or appropriate to, the given sex—the *stimulation of homologous characters;* and 2) *the inhibition of heterologous characters*, or those characters belonging to the opposite sex. Such inhibition of characters of the opposite sex would be extended even to the gonad.

A further point should be emphasized about hormones. Twenty-five years ago no effective preparations of sex hormones were available with which to experiment. Furthermore, at the present time when so many different pure chemical preparations, exhibiting properties of the natural hormones, are available we have no assurance a) that any of these are the same substances, or are in the same form of possible combination, as the secretions produced in the gonads, and b) that the hormones secreted by the gonads of sexually mature animals are the same as those that might be secreted by the embryonic gonad. A final general point, bluntly put, is that despite the enormous amount of investigation there is as yet no acceptable evidence that developing gonads secrete hormones at the stage in development when these hormones theoretically are acting to regulate sexual differentiation in the embryo.

With these preliminary ideas to guide our thinking and to aid us in arriving at opinions as to the meaning of a vast array of observations, I want to discuss some of the experiences of past work in our laboratories and in progress at the present time, as well as that being done in other laboratories.

Of several personal approaches already made, two separate phases may be mentioned briefly. With the aid of my colleague, Dr. Price (2), the

embryonic reproductive systems of female rats at known ages of gestation were dissected from the embryos and transplanted subcutaneously into males just after birth. By this procedure we hoped to closely duplicate the fundamental association present in the freemartin—i.e., to subject developing female systems to male hormones, if these were present in the male body. It would be expected that the developing ovary would be inhibited, the male ducts carried along with the transplanted female tissues would be stimulated, and the female duct system would be inhibited and caused to disappear. The differentiating female tract was not thus modified, however, but, while differentiating in the male body, the ovary and female ducts developed in a manner entirely similar to their development when transplanted into a female host.

Prior to the completion of investigation in which only the normal tissues of the body were concerned, instead of perhaps artificial hormonal agents of pure chemical type, an investigation was begun (3) in which the pure chemical androgen, testosterone propionate, was injected into pregnant rats of timed copulations, hence known periods in gestation, to study any possible effects that would be induced in the developing embryos. Procedures involved removal of one horn of the double uterus with its contained embryos one day preceding expected delivery (usually at 20 days+12 hours post-coitum) in order to preserve embryos; the remaining embryos were expected to be delivered and retained until they reached sexual maturity. But although relatively small doses of the chemical hormone were injected (highest dosage 5 mg. per day, 1 to 4 days) 60 per cent of the injected mothers showed injury to the placenta; in some cases three-fourths or more of all embryos present were hemorrhagic, dead, or undergoing resorption. This led us to question whether such drastic conditions would permit ordinary development to occur and suggested the question whether any abnormalities that might be found could be regarded as a specific effect of the hormones applied or were due to non-specific injuries on account of placental damage. To avoid these uncertainties the work in our laboratory was turned to the use of a mammalian form in which uncertainties relative to uterine injury would not enter the picture (4). The opossum, sole representative of the marsupial group of mammals on the North American Continent, supplied these needs. In the meantime Green, Burrill and Ivy (5–8) were actively studying these problems by use of the rat—other laboratories here and abroad had used mice, rats, chicks, and opossums (9–14)—and these investigators have extended their observations very widely on abnormalities resulting from such pre-natal treatments.

Green, Burrill and Ivy have described their materials and results extensively. A few special points and their implications may be given consideration. Injections of chemical androgenic substances or male hormones into pregnant mothers led to the retention in new-born females of the male duct system, so that both sets of sex ducts were present and well developed. Retention of the male ducts after androgenic treatment would be expected from the theory of hormonal control of reproductive development. The facts are undoubted and similar results were noted in rats treated in my laboratory. However, if the hormonal control theory provided a correct interpretation, the female sex ducts should have been inhibited and the ovary severely injured. These latter effects were not obtained since the oviducts and uterus were essentially normal, as was also the ovary. Furthermore, when pregnant females were treated with estrogenic substances, again the new-born females retained male ducts, which is the opposite of expectation and exactly contrary to the hormone theory of control of sexual differentiation.

In short, these and many other positive modifications obtained by this group of investigators by treatments of pregnant mothers with chemical hormones—facts that are not in question—make the explanation on the basis of the hormone theory an extremely difficult one unless assumptions are introduced for which there is lack of evidence, or evidence of a very questionable character.

A further important conception should be introduced here, namely, that although it be admitted that responses of sex ducts to introduced hormonal substances occur, this fact cannot be taken as evidence that the basic duct

systems require hormones for their development. Response during embryonic development can occur but this is not proof that such hormones are present during normal development and guide sexual differentiation.

The opossum, in many respects, presents exceptional material for such studies. The short period of approximately 13 days of gestation leads to birth of young which are very immature in development. At birth the young are approximately 10 mm. in total length, weigh about 0.1 gm., and are sexually undifferentiated. A firm attachment to nipples within the pouch is uninterrupted for approximately 60 days and the young can be treated with hormones by inunction.

In brief, when the young animal is passing through early stages in sexual differentiation, such as occur in placental mammals long before parturition, and is treated daily with hormones for whatever period may be selected, remarkable responses are to be seen. However, these responses are of such nature as to make it difficult to believe that the normal type of development is obtained through the action of similar hormonal influences; they fail to fit into the hormone conception. Male hormone preparations do not inhibit development of female structures, as would be expected on the basis of freemartin conditions, but rather the embryonic female sex ducts are tremendously stimulated; the ovary is not perceptibly affected. Female hormone substances applied to developing males did not inhibit male ducts and male gonad, as current theories would require, but rather the male ducts were stimulated. Especially striking was the demonstration that homologous organs in the two sexes possessed totally different capacities for response, which suggested that the basic genetic equipment of the two sexes was of farther-reaching consequence than were the applied hormones.

Thus in the analyses of these responses and in view of lack of acceptable evidence that the developing gonads actually secreted any hormones, it was tentatively suggested that development of the reproductive system did not depend upon hormones secreted by developing gonads and that possibly our present theories were incorrect (4).

A further step was directed towards an attempt to determine whether the gonad, during its early developing stages, actually secreted hormones or at what period such secretions were actually demonstrable. This attempt was based upon the conception, and assumption, that any gonad capable of secreting hormone would respond with precocious secretion when treated with gonad-stimulating agents; there is a great deal of evidence that this assumption is valid and nothing known to the writer is against it. Therefore, the young (males and females) were treated with the active sex-gland-stimulating principle from pregnant mare serum (equine gonadotropin) and killed at selected periods for study of the prostate gland in males and the uterine tract in females. If testes were capable of secreting, this treatment should increase male hormone secretion which should be detected by the response of the prostate gland; if ovaries were capable of secreting estrogens, the treatment should be detected by responses in the uterine tract. Actually, by using the prostate gland development as the indicator, secretions from the testicles were detectable for certain by the 70th day, and questionably by the 63rd day; by using differentiation in the female tract, secretions from the ovary were first detected after an age of 100 days (15): later studies in which Cowper's gland served as the male hormone indicator were able to extend detectable testicular responses to still earlier periods (16).

The conclusions to be drawn from these experiments would appear to be that gonads could be so stimulated, in the male by day 70 or earlier and in the female shortly after day 100, as to enable detection of gonad secretions by their action in precocious stimulation of sexual differentiation. However, this proof that the gonads could be so stimulated to secrete detectable amounts of hormone at these periods is not proof that they were actually secreting at such ages when no gonad-stimulating agent was applied. At these ages of detectable response following stimulating agents, the reproductive systems of both sexes were well developed. For hormones to have controlled the characteristic differentiation of the male and female tracts, they would of necessity have had to be secreted about day 10 and especially before day 30 when very characteristic differ-

ences between males and females are already established.

These facts strongly suggest, therefore, not only that early differentiation of the reproductive system is not controlled by gonadal-secreted hormones, but that gonads are not capable of secreting hormones until quite late periods in development; they presumably do not secrete normally until periods later than that at which they are capable of responding to gonadotropic agents.

The results of castration and spaying add further to the analysis of the problem. Successful gonadectomies on both sexes have been accomplished while the young were attached to nipples inside the marsupial pouch and as early as day 20 (17). In males, the prostate gland at this time is in the anlagen stage, represented only by small epithelial buds from urogenital sinus epithelium and in females the future reproductive tract is represented only by small straight luminate ducts; in each sex both male and female sex ducts are present at the earliest operation date.

Autopsies at selected periods up to 140 days of age show that in males the prostate gland, after castration, continues its differentiation and growth in characteristic manner; in comparison with normal controls, differences are not apparent until after 100 days of age when the prostate has advanced very far in its development. In ovariectomized females, the simple female ducts increase in size and, as in normal development, produce characteristic oviduct, uterus, cervix, lateral vaginal canal and urogenital sinus; uterine gland formation starts but is incomplete because of adhesions that develop as a result of operation. The first differences attributable to lack of hormone secretion are not apparent until later than 100 days of age (17). A similar condition attends development of such structures as Cowper's and Bartholin's glands (16).

These findings are interpreted to mean that, since sexual differentiation continues after removal of gonads in very early stages of development, the differentiation and development of the reproductive system up to comparatively complete systems does not depend upon hormones secreted by the developing gonads but is inherent in the genetic constitution of the genetically determined sexes; the hormone phase of action on the reproductive system appears to operate relatively late in sexual development, thus supplementing further development through the prepuberal and the puberal period; and sex hormones control the structure and function of the various parts during the reproductive age. When gonadal hormone secretion stops or is diminished—seasonally in some mammals and at the termination of a possible reproductive age in constantly breeding forms—reversions set in and typical involution of the reproductive system occurs.

It should be understood that this attempted analysis applies specifically to the opossum; how far it may apply to mammals in general awaits demonstrations of fact. In rats, the transplantation of reproductive tissues from 15-day old embryos into opposite-sexed hosts did not result in any modification of development that could be attributed to hormone actions. This introduces the question whether lack of response in early ages (late embryonic or early post-natal life) is a reflection of incapability of tissues to respond to androgens or estrogens, possibly secreted by gonads, or an actual lack of hormone secretion by the gonads which would affect the end-organs.

In the opossum, in which androgens and estrogens are applied to the embryo directly and without introducing the question of indirect effects because of placental injury, the sex ducts and accessory reproductive organs respond to estrogens and androgens at very early periods in differentiation; the prostate gland is markedly stimulated in males during the anlagen stage—in fact, androgens will induce prostate development in females, which do not produce such anlagen during normal development. The phallus and Cowper's gland likewise respond in very early stages of development. In females, similarly, the early undifferentiated female ducts respond to estrogens with precocious uterine development; the uterus is also remarkably stimulated prior to day 30 by treatments with androgens. Therefore, with capability of response of tissue demonstrated, the lack of responses until relatively late ages after gonadotropic treatment suggests that the gonads prior to such ages when hormone secretion

can be demonstrated are not yet secreting hormones. In the opossum the collective indications from several pieces of work involving injection of sex hormones, treatments with gonadotropic agents, development of various end-organs in both sexes after castration, and associated cytological phenomena in the pituitary (18) suggest that hormone secretion by gonads begins about day 100.

A somewhat roughly comparable condition in sexual differentiation in chicks has been shown by Willier and associates (13). Briefly portrayed, Willier was able to obtain rather marked responses in development of ducts and of gonads when the hormones were injected into the egg during early stages of development; modification towards intersexual conditions was noted. One might thus attribute to hormones far-reaching influences in normal development and in the control of sexual differentiation. However, Willier (19) had demonstrated earlier that if at the first appearance of a genital ridge the primordium of the system was transplanted into an animal of the opposite sex no modifications were induced in the differentiation in the graft or in the host. Willier arrived at the general conclusion that during these ages in development: a) either the host was not secreting sex hormones; or b) the hormones if secreted exerted no effect upon sexual differentiation of the reproductive tract. Since treatments with sexual hormones had proved the tissue capable of response and no response occurred when only normal tissue (and not chemical hormones) were employed, the conditions again suggest that the gonads were not secreting until relatively late periods in development—perhaps not until after the stage of hatching. Domm and Dennis (20) injected gonadotrophic substances into the developing chick egg and noted that embryos preserved on the 18th day possessed greatly hypertrophied gonads but no precocious development in sex ducts. The gonads apparently were not yet capable of secreting hormones, although shortly after hatching this capacity to secrete was clearly demonstrated (21).

A more extended discussion of these problems will be found elsewhere (22) but in the estimation of the writer it is strongly suggested: that the control of sex differentiation, up to the stage of a well-established reproductive system, rests upon the genetic sex-determining factors present in the embryo, which operate without the intervention of sex hormones secreted by developing gonads; that, in fact, the differentiating gonads do not secrete hormones until a period considerably later than that during proper disposition of the two sets of sex ducts, in accordance with the genetic determination of sex; and that when sex hormone secretion begins, the reproductive system is thrown into unusual activities in growth and development characteristic of the puberal ages. It is, of course, well established that sex hormones are responsible for the structural and functional control over the accessory reproductive organs during active sex life.

It is also well established both that the applications of androgens or estrogens to the organism during early post-natal and prepuberal life leads to precocious responses on the part of many of the accessory organs of reproduction and that similar responses of end-organs follow treatments with gonadotropic agents; in the first case androgens or estrogens are supplied from the outside and in the second, are caused to be secreted precociously by the intact gonads. In either case precocious puberal phenomena are evident, but when such treatments cease, development usually undergoes retrogression; normal puberty is not hastened, but in fact may be delayed by these precocious puberal phenomena induced experimentally.

SUMMARY

As a summarizing statement relative to the rôle of hormones in sex differentiation the writer's opinions may be expressed somewhat as follows.

Our current conceptions of the control of sex differentiation by hormones secreted by developing gonads is not substantiated by critical analyses of experimental results.

In some species it is demonstrated that removal of gonads prior to proper disposition of the two sets of embryonic sex ducts does not interfere with their proper differentiation to produce well defined male or female reproductive systems characteristic of the genetic sex determination. Sex duct differentiation, there-

fore, does not depend upon hormones secreted by the gonads.

Much evidence exists that differentiating gonads do not secrete sex hormones and are incapable in fact of doing so when treated with gonadotropic agents; no acceptable evidence is known to indicate sex hormone secretion by differentiating gonads.

Hermaphroditic conditions, such as are found in the human organism, are not believed to rest upon an hormonal basis for their establishment. When gonad tissue is present, and can secrete sex hormones, and when embryonic rudiments of the sex ducts or accessory organs of reproduction have been retained, hormonal responses enhancing the atypical sexual state can be expected.

REFERENCES

1. Lillie, F. R.: *J. Exper. Zool.* 23: 371. 1917.
2. Moore, C. R., and Dorothy Price: *J. Exper. Zool.* 90: 229. 1942.
3. Moore, C. R.: *Internat. de pharmacodyn et de therap.* 65: 365. 1941.
4. Moore, C. R.: *Physiol. Zool.* 14: 1. 1941.
5. Greene, R. R., M. W. Burrill and A. C. Ivy: *Am. J. Anat.* 65: 415. 1939.
6. Greene, R. R., M. W. Burrill and A. C. Ivy: *Anat. Rec.* 74: 429. 1939.
7. Greene, R. R., M. W. Burrill and A. C. Ivy: *Am. J. Anat.* 67: 305. 1940.
8. Greene, R. R., M. W. Burrill and A. C. Ivy: *Physiol. Zool.* 15: 1. 1942.
9. Hamilton, J. B., and J. M. Wolfe: *Anat. Rec.* 70: 433. 1938.
10. Raynaud, A.: *Bull. biol. de la France et de la Belgique* 72: 297. 1938.
11. Turner, C. D.: *J. Morphol.* 65: 353, 1939.
12. Wolfe, E., and A. Ginglinger: *Arch. d'anat., d'hist., et d'embryol.* 20: 219. 1935.
13. Willier, B. H., Mary Rawles, T. F. Gallagher and F. C. Koch: *Physiol. Zool.* 10: 101. 1937; *Proc. Nat. Acad. Sc.* 24: 176. 1938.
14. Burns, R. K., Jr.: *Jour. Morphol.* 65: 79 and 497. 1939. Cold Spring Harbor Symposia on Quant. Biol. 10: 27. 1942.
15. Moore, C. R., and C. F. Morgan: *Endocrinol.* 32: 17. 1943.
16. Rubin, D.: *J. Exper. Zool.* 94: 463. 1943.
17. Moore, C. R.: *J. Exper. Zool.* 94: 415. 1943.
18. Wheeler, R. S.: *J. Morphol.* 73: 43. 1943.
19. Willier, B. H.: *J. Exper. Zocl.* 46: 409. 1927.
20. Domm, L. V., and E. A. Dennis: *Proc. Exper. Biol. & Med.* 36: 766. 1937.
21. Domm, L. V.: Cold Spring Harbor Symposia on Quant. Biol. 5: 241. 1937.
22. Moore, C. R.: Amer. Nat. 78: 97. 1944.

Abstracts of
CURRENT ENDOCRINE LITERATURE

Editor: D. A. McGINTY. *Collaborators:* ISRAEL BRAM, RUCKER CLEVELAND, JOHN W. EVERETT, ANNE FORBES, ROBERT B. GREENBLATT, E. C. HAMBLEN, CHARLES W. HOOKER, R. G. HOSKINS, THOMAS H. MCGAVACK, A. E. MEYER, DORIS PHELPS, J. P. PRATT, E. D. REIFENSTEIN, JR., PATRICIA H. SMITH, RUTH ST. JOHN, CHARLES W. TURNER, HAROLD OOSTER.

BOOK REVIEWS

SELYE, HANS.

An Encyclopedia of Endocrinology, Section I "The Steroids." Published in 4 volumes by A. W. T. Franks, Publishing Company, Montreal, 1943.

This is the first section of an encyclopedia which it is proposed will encompass the entire field of endocrinology. As such it will be the first handbook on this subject published in the English language. Because endocrinology has such a wide scope this work should be of unusual interest not only to students of endocrine research but to all those interested in medical sciences in general. The author is well qualified to prepare such a work as he has wide experience in many aspects of experimental endocrinology. To facilitate the preparation of this handbook the author has collected over 150,000 reprints and abstracts on endocrine topics.

The present section, which is the first to appear, is a "Classified Index of the Steroid Hormones and Related Compounds." It is published in four attractive loose leaf binders. The choice of the loose leaf system is particularly suited for this section as it permits the addition of new sheets as further information becomes available and new compounds are prepared, and allows the rearrangement of the index to suit the individual needs of the reader.

The system of classification used in this index is based on chemical structure. It lists all the steroids as substitution products of saturated nuclear hydrocarbons. These are norestrane, estrane, D-homoestrane, pyroandrostane, 18-norandrostane, i-androstane, androstane, D-homoandrostane, etiocholane, 9-epietiocholane. By this method of classification pregnane, for example, becomes an alkyl substituted derivative of etiocholane. A separate page is devoted to each parent compound. In all 728 compounds are indexed. Each index page consists of the name of the compound, assigned according to the above classification, with the common name, if such exists, in parenthesis. Seven section headings appear on each page. They are: Isolation, Structure and Synthesis, Melting Point, Pharmacology, Remarks, Derivatives and References. Under each heading the information at present available concerning the compound is recorded and the requisite literature references given.

The index thus presents information which can be obtained from the original publications only at great trouble and with a considerable expenditure of time. The last such exhaustive classification appeared in "The Chemistry of the Sterids" by Dr. H. Sabotka of which no edition has appeared since 1938. It is of particular interest to pharmacologists and biochemists, but perhaps of less use to the organic chemist as the sterols, bile acids, etc., are not indexed.

This section of the encyclopedia contains several extremely useful synoptic tables. Perhaps of greatest interest is the synoptic chart which summarises all the information available regarding the hormonal activities of all the known biologically active steroids and their derivatives. This section alone makes the index an indispensable reference volume as many of the data herein recorded can be found in the original literature only with the utmost difficulty.

This section represents what must have been a long and tedious task for the author and he is to be congratulated on the excellence and completeness of this work. The book is indispensable to any laboratory in which the steroid hormones are studied, particularly since the author proposes to keep the index up to date by the periodic publication of supplementary sheets.—*J.S.L.B. and M.M.H.*

ADRENALS

CLINTON, M., AND G. W. THORNE

Effect of desoxycorticosterone acetate administration of plasma volume and electrolyte balance of normal human subjects. *Bull. Johns Hopkins Hosp.* 62: 255. 1943.

The effect of DOCA was studied in normals since it is well known that its administration induces a prompt and striking rise in plasma volume in patients with Addison's disease. Over a period of five to fifteen days 10 milligrams of DOCA was given intramuscularly and an increase in plasma volume was noted in all four normal subjects. The increase persisted throughout the period of treatment and fell to normal or slightly below within three days after its withdrawal. Sublingual administration of 30 to 40 milligrams of DOCA in divided doses was less effective than 10 milligrams once daily intramuscularly. Supplementary sodium chloride given simultaneously did not augment the increase in plasma volume following the administration of DOCA; however, the total sodium, chloride, and water retained during the period of treatment with DOCA was increased appreciably. Supplementary sodium chloride alone was followed by a slight increase in plasma volume. No evidence of toxic symptoms was observed during the five to fifteen days in which DOCA was given.—*P.H.S.*

SHIPLEY, R. A.

Treatment of Addision's disease with pellets of desoxycorticosterone acetate. *Am. J. Med. Sci.* 207: 19. 1944.

The author treated 7 patients with pellets ranging in size from 75 mg. (cylinders) to 150 mg. (flat discs). Five patients were well maintained for 7 to 40 months and were able to perform work involving moderate physical activity: one of these died suddenly (perhaps of hypoglycemia) after an attack of appendicitis at a time when he seemed to be convalescing satisfactorily. The other two patients were not satisfactorily controlled by desoxycorticosterone in any dosage by pellets or by injection, and died from the disease. Pellets weighing 75 mg. approach complete absorption more slowly than those that are larger and, therefore, are more satisfactory. The effective life of the 75 mg. pellet (Schering) which is being used at present is approximately 9 to 10 months, the average daily adsorption is 0.21 mg. per pellet, and the average number of pellets required is 4 to 6. The rate of absorption is reasonably constant from patient to patient and from pellet to pellet. Symptoms of over-dosage requiring removal of pellets occurred only once in a patient in whom the number of pellets was increased for special reasons. The author stresses that: 1) it is possible to implant pellets without subjecting the patient to a lengthy preliminary period of injection therapy; 2) patients under therapy with pellets are easily thrown into hypoglycemia by fasting or infection; 3) desoxycorticosterone therapy in addition to its failure to restore normal carbohydrate metabolism is deficient in other respects, and, therefore, cannot control the symptoms of the disease in all cases; and 4) during infections and other conditions imposing stress the requirement for hormone is increased and additional therapy chiefly in the form of cortical extract should be given. He concluded that pellet therapy with desoxycorticosterone acetate is highly useful for the maintenance of most patients with Addison's disease. —*E.C.R., Jr.*

SOFFER, L. J., G. LESNICK, S. Z. SORKIN, H. H. SOBOTKA AND M. JACOBS

The utilization of intravenously injected salt in normals and in patients with Cushing's syndrome before and after administration of desoxycorticosterone acetate. *J. Clin. Invest.*, 23: 51. 1944.

The subjects of this study were 12 normal individuals, and 5 patients with clinical evidences of Cushing's syndrome, from one of whom an adrenocortical tumor was subsequently removed. The influence of desoxycorticosterone acetate on salt utilization was determined by the following procedure: Except as later indicated, the subject was permitted neither fluid nor food from 7 p.m. the evening before the test until the test period was completed. At 6 a.m. on the morning of the studies, 500 cc. of water were ingested at one time, and the urine from that moment until 9 a.m. was analyzed for its total volume and its sodium and chloride content. At 9 a.m., 200 cc. of 5 per cent saline was administered intravenously, and the urine excreted from then until 12 noon was treated in a fashion similar to that described for the earlier period. After a 2-day rest, the entire procedure was repeated following a dose of 10 mgm. DCA administered at 10:30 p.m. on the preceding evening. From these data the percentage of the injected sodium and chloride ions excreted in the 3 test periods was calculated. As a result, it was found that the injection

of DCA in normal individuals caused a decreased excretion of sodium and chloride as compared with the normal, the actual percentual change varying in different individuals from as little as 11 to more than 100 per cent as compared with the "normal" or control test. On the contrary, in all 5 of the patients with Cushing's syndrome, there was an increase in the urinary excretion of sodium and chloride ions following the administration of DCA. This increase reckoned percentually varied from 32 to 280. Possible mechanisms involved in the altered response of the patient with Cushing's syndrome were discussed. The authors have proposed the test as of possible value in the diagnosis of hyperfunction of the adrenal cortex.—*T.H.McG.*

WILHELM, S. F., AND S. GROSS

Surgical removal of adrenal adenoma with relief of Cushing syndrome, *Am. J. Med. Sci.* 207: 196. 1944.

A 46 year old man during the course of 1½ years developed: 1) marked adiposity of the face, neck and abdomen with thin extremities; 2) dusky redness of the face and neck; 3) purplish striae on abdomen; 4) headaches, dizziness, blurring of vision, poor memory, irritability and hypertension (190/118); 5) impotence and small, flabby testes; 6) diabetes; 7) osteoporosis of the skull; 8) markedly elevated urinary 17-ketosteroid excretion (93 mg./24 hr.); 9) normal urinary estrogen excretion (11 gamma/24 hr. calculated as estrone); and 10) a mass in the right suprarenal region demonstrated by excretion pyelographic studies and confirmed by perirenal insufflation and laminography. Because of the normal estrogen excretion a benign tumor was suspected. At operation a non-malignant adenoma of the right adrenal weighing 284 grams was removed. Post-operatively the patient had a stormy course complicated by: 1) infection of the wound and cellulitis that was twice incised; 2) inflammation of abdominal wall fatty tissue which required a laparotomy and excision on the 44th day; 3) phlebitis on the 53rd day; and 4) oliguria and severe anasarca on the 7th to 12th post-operative day from too-energetic treatment with adrenal cortical extract, desoxycorticosterone, testosterone, and saline infusions. The latter responded quickly to discontinuance of this therapy and to the prompt administration of mercupurin, potassium chloride, and 5% glucose in distilled water. The patient left the hospital improved on the 67th day after adrenalectomy. He has remained well for 16 months since operation; blood pressure is normal (120/80); redness, striae, and obesity have disappeared; sexual potency has returned, and testes are larger and firmer; urinary 17-ketosteroid excretion has become normal (15 to 20 mg./24 hr.).—*E.C.R., Jr.*

ENDOCRINE GENERAL

COHN, E. W., AND J. H. ROE

A new technique for the measurement of glycogenesis: a study of glycogen metabolism in the liver under fasting conditions. *J. Lab. & Clin. Med.*, 29: 106. 1944.

The authors' method for measuring glycogenesis involves the use of double laparotomy under nembutal anesthesia. At the first operation a sample of liver is obtained from the animal while fasting. This serves as a control. The second operation is performed 6 to 12 hours later, after the test substance has been used. The influence of the operative procedure itself and that of nembutal anesthesia upon glycogenesis have been determined and base-line values calculated for correction of results obtained by the authors' technique. An appraisal of the effect of preoperative fasting has been made, as a result of which the authors advise using animals at from 24 to 36 hours after food has been withdrawn. A study of the action of dextrose and mannoheptulotose on glycogenesis is included in the report by way of illustration. The results were "highly significant."—*T.H.McG.*

DARROW, D. C., AND E. L. SARASON

Some effects of low atmospheric pressure on rats. *J. Clin. Invest.*, 23: 11. 1944.

Rats of Wistar strain weighing between 200 and 250 gm. were exposed to air pressures equivalent to elevations of 20,000 and 25,000 feet for periods up to 7 days. Groups of normal rats, rats on a low potassium diet, and rats receiving desoxycorticosterone acetate for 14 days before and during exposure to low atmospheric pressures were studied. The animals receiving DCA failed to become acclimatized at 20,000 feet, and none was able to survive for 24 hours at 25,000. There were no deaths in the other groups of animals at either altitude, although none of the animals ever adjusted fully to conditions at the 25,000 foot level. No significant changes in adrenal weight were observed except in rats on a low potassium diet, kept at altitudes of 25,000 feet for from 1 to 2 days. In these a definite increase was noted. All groups showed a depletion

of adrenocortical lipids beginning as early as 2 hours after exposure to low atmospheric pressures. At 20,000 feet, in 1 to 2 days the reaction was definite in normal rats, more noticeable in those receiving a low potassium diet, and most striking in those given DCA. All animals that survived for 7 days showed a return to normal of adrenocortical lipids which, in the author's opinion, was remarkable in view of the inability of any one of the groups of animals to become fully acclimatized at 25,000 feet. The kidneys were relatively large in the animals on a low potassium diet and in those receiving DCA. Diminished oxygen tension played no part in this hypertrophy. Cardiac lesions could be demonstrated only in animals receiving DCA. The usual unfavorable response of the heart of the normal animal at sea level to overdosage with DCA was markedly increased by a lowered oxygen tension. Similar treatment of the potassium group was carried out without the demonstration of microscopic lesions, which leads to the conclusion that the depletion of body stores of potassium is not the sole cause of these lesions. DCA was found to aggravate the accumulation of intracellular sodium which may be produced in normal rats by anoxemia. Values for muscle potassium were low in normal animals fasted 7 days and in those exposed to atmospheric pressures equivalent to an altitude of 25,000 feet (attributed to voluntary fasting). These low values showed no further downward alteration under the influence of DCA, despite its action in raising intracellular sodium. The liver was frequently enlarged as a result of low atmospheric pressures in all groups of animals, but changes in the composition of the liver were not altered by altitude equivalents of 20,000 and 25,000 feet.—*T.H.McG.*

HUDGINS, A. P.

Stilbestrol in the treatment of the extrauterine placenta. *South. M. J.* 36: 678. 1944.

This paper describes how the use of from 25 to 50 mg. of stilbestrol daily after removal of the fetus from an abdominal pregnancy minimized bleeding from the left-in abdominal placenta and facilitated later surgical removal.—*H.A.W.*

THYROID

HAMILTON, J. G., M. H. SOLEY, W. A. REILLY AND K. B. EICHORN

Radioactive iodine studies in childhood hypothyroidism. *Am. J. Dis. Child.* 66: 495. 1943.

The authors have studied the uptake of radioactive iodine in 8 children (ranging in age from 3½ to 18 years) with hypothyroidism without goiter, and in 2 children (age 4½ and 19 years) with severe hypothyroidism and goiter. The thyroids of the non-goitrous children were found to concentrate smaller amounts of orally administered iodine than the thyroids of normal children or adults; in these hypothyroid children, the remaining thyroid tissue concentrated a larger percentage of a dose of 0.1 microgram than a dose of 14 milligrams of iodine. The authors pointed out that by the use of extremely small amounts of radioiodine the presence of thyroid tissue may be detected in patients with hypothyroidism or myxedema. The thyroids of the goitrous children took up relatively large amounts of iodine in a manner similar to the thyroids of adults with hyperthyroidism. By chemical analysis the thyroids of these goitrous children contained both thyroxin and diiodotyrosine in considerable quantities; radioautographs of sections from these glands indicated that by the fifth day after oral administration more of the iodine was in the cells than in the colloid. The authors suggest that the metabolic fault in these goitrous hypothyroid children may lie in the inability of the thyroid to release rather than to produce thyroxin. They point out that the extremes of thyroid function exhibit certain similarities susceptible to no further interpretation today than when they were first discovered many years ago by David Marine.—*E.C.R., Jr.*

HINTON, J. W., E. B. ECKERSON AND M. BRUGER

Iodine metabolism in thyroid diasese. Clinical and experimental observations. *Ann. Surg.* 115: 206. 1942.

1. The blood iodine, thyroid gland iodine and histology of the thyroid were studied in guinea pigs following the administration of equivalent amounts of iodine in the form of potassium iodide, sodium iodide, diiodotyrosine, thyroglobulin and thyroxine. Potassium iodide, sodium iodide and diiodotyrosine elevated both the blood and thyroid gland iodine without altering the histologic picture of the thyroid gland. Thyroglobulin and thyroxine also increased the blood and thyroid gland iodine but this was associated with evidence of increased activity of the acinal cells. 2. Similar studies were carried out following the administration of thyrotropic factor of the anterior pituitary, which contains no iodine. Guinea pigs treated with this substance showed a decrease in the thyroid gland iodine without any change in the blood iodine, marked stimulation of cellular activity and diminution of the

colloid content of the acini. 3. Determinations of the total blood iodine were carried out on 236 patients who had, or were suspected of having, thyroid disease. None of the patients had been taking iodine-containing compounds within one month of the blood studies. Approximately 25% of 65 patients with non-thyroid disorders had an elevated blood iodine, a finding attributed to unsuspected exposure to iodine. Approximately 80% of 66 patients with exophthalmic goiter and 50% of 19 patients with toxic nodular goiter showed an elevated blood iodine. Contrary to the findings of others, the level of blood iodine in patients with exophthalmic goiter bore no relation to the duration of the disease. In persistent or recurrent exophthalmic goiter (8 cases of each), the incidence of an elevated blood iodine was no greater than in patients with non-thyroid disease. It is stated, "Clinical experience in addition to blood iodine studies has helped to formulate the opinion that in many patients with persistent or recurrent exophthalmic goiter, the diagnosis of hyperthyroidism, which led to surgery, was probably erroneous and that the decreased iodine content of the blood was not indicative of the exhaustion of the thyroid gland."—*D.P.*

HIMSWORTH, H. P.

Thyrotoxicosis treated with thiourea. *Lancet* 245: 465. 1943.

Thiourea has a beneficial effect on thyrotoxicosis, probably by interfering with synthesis of the thyroid hormone. Its only apparent disadvantages are an emetic effect which disappears on further treatment, and a peculiar sweet smell of the breath which is more unpleasant to the observer than to the patient. Six cases were treated, of which 1 is presented in detail. Two gm. per day is an adequate maintenance dose; no relapses have occurred.—*Courtesy Biol. Absts.*

LA DUE, J. S.

Myxedema heart: a pathological and therapeutic study. *Ann. Int. Med.* 18: 332. 1943.

Postmortem findings in the heart of a patient who died of heart failure due to myxedema revealed hydropic vacuolization, loss of striation, branching, pyknotic nuclei and irregularity in staining properties of the muscle fibrils. The author claims that these findings are not specific for myxedema heart as they have been demonstrated in beriberi heart and in the heart of patients dying of other than cardiac deaths. He states that thiamin and vitamin B concentrate are effective in the treatment of beriberi heart but do not improve the myxedema heart which responds to thyroid medication.—*M.B.G.*

LAHEY, F. H., L. M. HURXTHAL AND R. E. DRISCOLL

Thyrocardiac disease. A review of 614 cases. *Ann. Surg.* 118: 681. 1943.

The term thyrocardiac has been applied by Lahey to patient in whom early diagnosis and early subtotal thyroidectomy can accomplish gratifying restoration of cardiac capacity and physical activity. In this review of the authors' experience with the disease, advances in treatment are summarized and the hope that, in future, earlier diagnosis and operation and even prophylactic subtotal thyroidectomy for early toxic adenoma and early auricular fibrillation will give even better results is expressed. The necessity of speaking of a thyrocardiac as one having indisputable hyperthyroidism is stressed. Aspects of the subject discussed include 1) the reason the true thyrocardiac permanently regains cardiac compensation after subtotal thyroidectomy while the false thyrocardiac does not, 2) the preoperative and operative management of the disease and 3) the authors' experience with various types of anesthesia. The end-results in 614 cases, from 1923 to 1933 and from 1933 to 1943 are presented. Of the 614 patients, 306 are known to be alive and 165 are known to be dead. Of those known to be alive, 201 are able to carry on normal activities commensurate with individual age, and have regular heart rhythm, no myxedema and no recurrence of hyperthyroidism, 100 carry on normal activities but still have auricular fibrillation or postoperative myxedema requiring thyroid or have had a recurrence of hyperthyroidism controlled by iodine and 5 are incapacitated by some complication attributable to their thyrocardiac condition. The incidence of postoperative myxedema for the series is 2.9%, the incidence of diabetes, 3.6%.—*D.P.*